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Test Methods for Nonconventional Pesticides Chemicals Analysis of Industrial and Municipal Wastewater

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Effluent Guidelines Division
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January 31, 1983

Water and Waste Management

PB83-176636

EPA

Test Methods

Methods for Nonconventional Pesticides Chemicals Analysis of Industrial and Municipal Wastewater

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16. ABSTRACT

This document presents the findsings of studies of the pesticide chemicals point source category for the purpose of developing test methods for the analysis of 66 of the 137 nonconventional pesticide pollutants for which effluent limitations guidelines and standards were proposed on November 30, 1982. These test methods are proposed under authority of Sections 304(h) and 501(a) of the Clean Water Act, 33 U.S.C. 1251 et. 5eg. (the Federal Water Pollution Control Act Amendments of 1972) (the "Act"). This document contain compilation of three sets of methods: those developed by industry, those developed by the contractor and those developed/approved by EMSL. Quality assurance (QA) requirements for the collection, preservation and handling of samples and requirements of quality control (QC) and safety are also contained in this report. Supporting data and rationale for the test methods are contained in this report and supporting file records.

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METHODS FOR NONCONVENTIONAL PESTICIDE ANALYSIS OF INDUSTRIAL AND MUNICIPAL WASTEWATER

U.S. ENVIRONMENTAL PROTECTION AGENCY

George M. Jett
Project Officer

January 31, 1983

Disclaimer

This report has been reviewed by the Effluent Guidelines Division, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

U.S. Environmental Protection Agency
Effluent Guidelines Division
Waterside Mall (WH-552)
401 M. Street, S.W.
Washington, D.C. 20460

Foreword

The Environmental Protection Agency proposes to establish test procedures for the analysis of 66 nonconventional pesticide pollutants for which effluent limitations guidelines and standards were proposed on November 30, 1982, in 40 CFR Part 455 (47 FR 53994). These procedures will be used in supporting the effluent limitations guidelines and standards proposed in 47 FR 53994 and would also be used for filing applications for the National Pollutant Discharge Elimination System (NPDES) permits, for state certification, and for compliance monitoring under the Clean Water Act.

Under the authority of Sections 304(h) and 501(a) of the Clean Water Act, 33 U.S.C. 1251 et seq (the Federal Water Pollution Control Act Amendments of 1972, as amended by the Clean Water Act of 1977) the Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants and is authorized to prescribe such regulations as are necessary to carry out its functions under this Act.

The test procedures in this document are a compilation of three sets of analytical methods: those developed by the Pesticide Industry found in Part A, those developed by the Agency contractors found in Part B, and those developed/approved by the Agency's Environmental Monitoring and Support Laboratory--Cincinnati (EMSL--Ci) found in Part C. All methods contained herein follow an Agency specified format while still maintaining the integrity of the original method.

A fourth section of this document (Part D) contains Agency requirements for sample collection, preservation, and handling; quality control; and safety. These requirements were designed for universal use and can be applied to each method found in Parts A, B, and C of this document.

Jeffery D. Denit, Director
Effluent Guidelines Division

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This document was developed under the supervision and guidance of Mr. George M. Jett, who served as project officer for the development of the November 30, 1982 pesticide technical regulations and the January 1983 pesticide analytical regulation. Other Agency key personnel who assisted on this document were Mr. Devereaux Barnes, Ms. Nancy Parkinson, Mr. S. Scott Saavedra and Ms Susan Schmedes.

Additional technical assistance was provided by Dr. James Longbottom, Dr. James Lichtenberg, Dr. Robert Booth, and Mr Edward Kerns of the Environmental Monitoring and Support Laboratory in Cincinnati, Ohio.

This document was prepared by Environmental Science and Engineering, Inc., (ESE) under contract number 68-01-6701. The project was managed by Ms. Barbara Brown, under the direction of Mr. James B. Cowart. Technical guidance and direction were provided by Dr. John Mousa and Mr. Stuart Whitlock. Key personnel included Dr. Dilna Victor, Ms. Karen Hatfield, Mr. Peter Beck, and Ms. Paula Anderson. Ms. Suzanne Albrecht coordinated the production of the document, which was edited by Ms. Patricia McGhee and typed in it entirety by Ms. Carolyn Granger. Analytical methods were provided by ESE and two additional contractors, whose efforts are acknowledged as follows: Dr. Herbert C. Miller and Ms. Ruby James of Southern Research Institute: Mr. John Clausen, Mr. Bob Beimer, Mr. Mike O'Rell and Mr. Bill Coleman of TRW Systems, Inc.

Acknowledgement is also made of the cooperation of personnel in many plants in the pesticide chemicals industry who provided valuable data and information relating to nonconventional pesticide analytical methods developed and used by their companies.

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Appendix B. Abbreviations, Acronyms, and Other Terms Used in this Document

Introduction

Under the Clean Water Act, the Agency is required to regulate three broad categories of pollutants. These categories are toxic pollutants, conventional pollutants, and nonconventional pollutants. Toxic pollutants are a defined list of 126 pollutants derived from the NRDC Consent Decree, 8 ERC 2120 (D.D.C. 1976), modified 12 ERC 1833 (D.D.C. 1979). Conventional pollutants are biological oxygen demand (BOD), total suspended solids (TSS), oil and grease, fecal coliform, and pH. A nonconventional pollutant is any pollutant not identified as a toxic pollutant (Section 307(a)(1) of the Act) or as a conventional pollutant (Section 304(a)(4) of the Act).

Section 304(h) of the Clean Water Act directs the Agency to approve analytical methods for the analysis of pollutants. These methods are necessary for filing applications for the National Pollutant Discharge Elimination System (NPDES) permits, for state certifications, and for compliance monitoring under the Clean Water Act.

On November 30, 1982, the Agency proposed effluent limitations guidelines and standards for 34 specific toxic pollutants, BOD, COD, TSS, pH, and 137 nonconventional pesticide pollutants in the Pesticide Chemicals Industry (47 FR 53994). An integral part of the effluent limitations guidelines and standards for the Pesticides Industry is the regulation proposing approved test procedures. To date, EPA has established analytical methods for only 49 of the nonconventional pollutants regulated by the proposed pesticide effluent limitations guidelines and standards. EPA projected that additional nonconventional pesticide analytical methods would be proposed by January 1983. In support of these regulations the Agency has compiled test procedures for an additional 66 nonconventional pesticide pollutants.

The Agency is proposing nonconventional pesticide analytical methods for 66 of the 137 nonconventional pesticide pollutants for which Agency-approved procedures do not currently exist. The remaining 71 nonconventional pesticide pollutants regulated in the November 30, 1982 document proposing effluent limitations guidelines and standards for the Pesticide Industry have either 304(h) approved methods or require no method since EPA proposed to establish "no discharge of process wastewater" as the applicable regulation.

The January 1983 proposed rulemaking (Guidelines Establishing Test Procedures for the Analysis of Nonconventional Pesticide Pollutants in the Pesticide Industry) includes a compilation of three sets of methods: those developed/used by the industry, those developed/used by Agency contractors, and those developed/approved by the Environmental Monitoring and Support Laboratory in Cincinnati (EMSL-Ci). The Agency is proposing to incorporate by reference these test procedures for nonconventional pesticide pollutants in 40 CFR Part 455 Section 170 pursuant to Subpart 401.13.

This document includes the abovementioned methods and is divided into four parts. Part A includes 45 industry-developed/used methods involving such instrumental techniques as gas chromatography (GC), spectrophotometry, high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography/mass spectrometry (GC/MS), and titration and are numbered 101 through 145. Part B includes nine contractor-developed/used methods including GC, spectrophotometric, HPLC, and GC/MS methods and are numbered 401 through 409. Part C includes 15 EMSL-developed/approved methods such as GC, GC/MS, HPLC, and spectrophotometric methods and are numbered 604, 608.1, 614, 615, 617, 619, 622, 625, 627, 629 to 633 and 701. In addition to analytical

methodology, Section D contains quality assurance (QA) requirements for collection, preservation, and handling of samples; quality control (QC); and safety. These requirements are applicable to all methods enclosed in this document. The EMSL procedure for determining the method detection limit is presented in Appendix A. Appendix B lists abbreviations, acronyms, and other terms used in this document.

The industry and contractor methods contained herein have been assembled into a format consistent with the EMSL-developed methods. This effort was completed for ease of reading and consistency; however, the integrity of the original method is still maintained. The methods in their original form are referenced at the end of each procedure.

Storet and CAS numbers were not available for all nonconventional pesticides identified in this document (see Scope and Application section of each method). Therefore, an errata sheet will be prepared at a later date when these numbers become available.

Only the 66 nonconventional pesticide pollutants previously mentioned are included in the methods titles; however, both these pollutants and others which can be analyzed by each method are listed under the "scope and application" of all methods presented in this document. The methods in Part A were developed/used by various members of the Pesticide Industry to generate data which were supplied to the Agency in response to information requests made under the authority of Section 308 of the Act. The methods in Part B were developed/used by the Agency contractors during the Pesticides Industry Verification Program. The methods in Part C were developed/approved by the Agency's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. The EMSL methods were not used for data generation for the proposed pesticide effluent limitations and standards since data were collected prior to their development.

In some cases methods from three sources (industry, contractor, and EMSL) are available for one nonconventional pesticide pollutant (see Table 1A). The Agency is presenting all available methods for each nonconventional pesticide. The Agency then intends to select the most appropriate methods for promulgation.

References

1. National Resources Defense Council, Inc., et al., v. Train, 8 ERC 2120 (D.D.C. 1976), modified 12 ERC (D.D.C. 1979).
2. Guidelines Establishing Test Procedures for the Analysis of Pollutants, 40 Code of Federal Regulations (CFR), Part 136, Published in Federal Register, 44, 69464.
3. "Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater," July 1982, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268 (EPA-600/4-82-057).
4. Pesticide Chemicals Category Effluent Limitations Guidelines, Pretreatment Standards, and New Source Performance Standards, 40 CFR, Part 455, Published in Federal Register, 47, 53994.
5. "Development Document for Effluent Limitations Guidelines and Standards for the Pesticides Point Source Category," November 1982, U.S. Environmental Protection Agency Effluent Guidelines Division, Washington, D.C. 20460 (EPA-440/1-82/079-b).

Proposed Test Procedures for Nonconventional Pesticides

Table 1A provides a list of proposed test procedures for 66 nonconventional pesticide pollutants proposed for regulation in 40 CFR Part 455 (47 FR 53994).

Table 1A. List of Proposed Test Procedures for Nonconventional Pesticides

Nonconventional Pesticide	Industry "100-Series" (Method Type)	Contractor "400-Series" (Method Type)	EMSL "600-Series" and "700-Series" (Method Type)
1. Alachlor	101,102(GC)	None	None
2. AOP	103(SPM)	401(SPM)	630(SPM)
3. Benfluralin	104(GC)	None	627(GC)
4. Benomyl	105,106(HPLC)	402(HPLC)	631(HPLC)
5. Bentazon	107(GC)	None	None
6. Bolstar	108(GC)	None	622(GC)
7. Bromacil	109(HPLC),141(GC/MS)	None	633(GC)
8. Busan 40	110(SPM)	None	630(SPM)
9. Busan 85	110(SPM)	None	630(SPM)
10. Butachlor	101,102(GC)	None	None
11. Carbam-S	None	None	630(SPM)
12. Carbendazim	105,106(HPLC)	402(HPLC)	631(HPLC)
13. Carbofuran	111(GC)	403(HPLC)	632(HPLC)
14. Carbophenothion	None	None	617(GC;GC/MS Cf),701(GC)
15. Chlorobenzilate	112(TLC)	404(HPLC)	608.1(GC;GC/MS Cf)
16. Chloropyrifos	113(GC)	None	622(GC)
17. Chloropyrifos methyl	113(GC)	None	622(GC)
18. Coumaphos	114(GC)	None	622(GC)

GC = Gas chromatography
SPM = Spectrophotometry
HPLC = High pressure liquid chromatography
GC/MS = Gas chromatography/mass spectrometry
Cf = Confirmation
TLC = Thin layer chromatography

Table 1A. List of Proposed Test Procedures for Nonconventional Pesticides (Continued)

Nonconventional Pesticide	Industry "100-Series" (Method Type)	Contractor "400-Series" (Method Type)	EMSL "600-Series" and "700-Series" (Method Type)
19. Cyanazine	115(TLC),116(HPLC)	409(HPLC)	629(HPLC)
20. 2,4-DB	117(GC)	405(GC)	615(GC)
21. 2,4-DB isobutyl ester	None	405(GC)	615(GC)
22. 2,4-DB isooctyl ester	None	405(GC)	615(GC)
23. DBCP	None	None	608.1(GC;GC/MS Cf)
24. Deet	118(GC)	None	633(GC)
25. Dichlofenthion	None	None	701(GC)
26. Dichlorvos	119,120(GC)	None	622(GC)
27. Dinoseb	121,122(GC)	406(GC),407(GC/MS)	615(GC)
28. Dioxathion	None	None	701(GC)
29. Ethalfluralin	104(GC)	None	627(GC)
30. Ethion	123(GC)	None	614(GC;GC/MS Cf)
31. Etridiazole	124(GC)	None	608.1(GC;GC/MS Cf)
32. Fensulfothion	125(GC)	None	622(GC)
33. Fenthion	126(GC)	None	622(GC)
34. Ferbam	103(SPM)	401(SPM)	630(SPM)
35. Fluometuron	144(TLC)	None	632(HPLC)
36. Glyphosate	127,140(HPLC)	None	None
37. Hexazinone	109(HPLC),141(GC/MS)	None	633(GC)

TLC = Thin layer chromatography
HPLC = High pressure liquid chromatography
GC = Gas chromatography
GC/MS = Gas chromatography/mass spectrometry
Cf = Confirmation
SPM = Spectrophotometry

Table 1A. List of Proposed Test Procedures for Nonconventional Pesticide (Continued)

Nonconventional Pesticide	Industry "100-Series" (Method Type)	Contractor "400-Series" (Method Type)	EMSL "600-Series" and "700-Series" (Method Type)
38. Isopropalin	104(GC)	None	627(GC)
39. KN-Methyl	110(SPM)	None	630(SPM)
40. Mancozeb	128(GC)	None	630(SPM)
41. Maneb	129(SPM)	None	630(SPM)
42. Mephosfolan	130(GC)	None	None
43. Metham	131(GC)	None	630(SPM)
44. Methomyl	109(HPLC),132(HPLC),133(GC)	408(HPLC)	632(HPLC)
45. Metribuzin	145(HPLC)	None	633(GC)
46. Mevinphos	119,120,134(GC)	None	622(GC)
47. Nabam	None	None	630(SPM)
48. Naled	119,120(GC)	None	622(GC)
49. Niacide	103(SPM)	401(SPM)	630(SPM)
50. Oxamyl	109(HPLC)	None	632(HPLC)
51. PCP Salt	None	None	604(GC),625(GC/MS)
52. Phorate	130(GC)	None	622(GC)
53. Profluralin	135(TLC)	404(GC)	627(GC)
54. Propachlor	101,102,143(GC)	None	608.1(GC;GC/MS Cf)
55. Ronnel	None	None	622(GC)
56. Simetryne	136(TLC)	None	619(GC)

GC = Gas chromatography
SPM = Spectrophotometry
HPLC = High pressure liquid chromatrography
GC/MS = Gas chromatography/mass spectrometry
Cf = Confirmation
TLC = Thin layer chromatography

Table 1A. List of Proposed Test Procedures for Nonconventional Pesticides (Continued)

Nonconventional Pesticide	Industry "100-Series" (Method Type)	Contractor "400-Series" (Method Type)	EMSL "600-Series" and "700-Series" (Method Type)
57. Stirofos	116(HPLC),119(HPLC),120(GC)	None	622(GC)
58. Terbacil	109(HPLC),141(GC/MS)	None	633(GC)
59. Terbufos	130(GC)	None	None
60. Terbutryn	None	404(GC)	619(GC)
61. Triadimefon	137(TLC)	None	633(GC)
62. Trichloronate	138(GC)	None	622(GC)
63. Tricyclazole	139(HPLC)	None	633(GC)
64. ZAC	103(SPM)	401(SPM)	630(SPM)
65. Zineb	103(SPM)	401(SPM)	630(SPM)
66. Ziram	103(SPM),142(TTN)	401(SPM)	630(SPM)

HPLC = High pressure liquid chromatography
GC = Gas chromatography
GC/MS = Gas chromatography/mass spectrometry
TLC = Thin layer chromatography
SPM = Spectrophotometry
TTN = Titration

PART A

INDUSTRY ANALYTICAL METHODS

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF ALACHLOR, BUTACHLOR, AND PROPACHLOR IN WASTEWATER

METHOD 101

1. Scope and Application

1.1 This method covers the determination of certain acetanilides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Alachlor	77825	15972-60-8
Butachlor	77860	23184-66-9
Propachlor	77729	1918-16-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in wastewater streams.

1.3 The method detection limit (MDL) for each parameter is 0.5 ug/g (ppm).

2. Summary of Method

2.1 An aliquot of wastewater (100 g) is extracted with chloroform. The extract is drained through a wad of cotton and collected in a vial containing 2 mL of internal standard solution. The chloroform extract is concentrated and analyzed by GC with flame ionization detector.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Glenco liquid syringe 5 or 10 mL or equivalent.

5.3 Hewlett Packard 5830 A Gas Chromatograph or Equivalent

5.4 Hewlett Packard 18850 A GC Terminal or Equivalent

6. Reagents

6.1 N,N-Dibutylaniline, Eastman Kodak Co. Reagent grade or equivalent

6.2 Chloroform, certified ACS, Fisher Scientific company or equivalent

6.3 Alachlor (Lasso), Monsanto Co., known % purity

6.4 Propachlor (Ramrod), Monsanto Co., known % purity

6.5 Butachlor (Machete), Monsanto Co., known % purity

7. Calibration

7.1 GC Conditions

GC Conditions are summarized in Table 1.

7.2 Calibration Procedure

7.2.1 Into a 100-mL volumetric flask weigh 0.1000 g of each expected pollutant (Alachlor, Butachlor, and Propachlor).

7.2.2 Dilute to mark with chloroform.

7.2.3 Make a further dilution by diluting 10 mL of this solution to 100 mL in a 100-mL volumetric flask. This solution will be used to make up calibration standards.

7.2.4 Into a 100-mL volumetric flask place one small drop of N,N-dibutylaniline and dilute to mark with chloroform. This will be the internal standard solution for calibration standards and samples.

7.2.5 Into a 4-dram vial pipet 2 mL of the internal standard solution.

7.2.6 Into the same 4-dram vial pipet 10 mL of the calibration standard.

7.2.7 On a hot plate evaporate this solution to 0.5 mL using moderate heat and a jet of air.

7.2.8 Place concentrated standard in a 1-mL serum cap injection vial (Teflon-lined cap).

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Weigh 100 g of waste sample into a 125-mL separatory funnel.

10.2 Using four 10-mL aliquots of chloroform extract the organics from the water. Shake for one minute each time and allow complete separation of the layers.

10.3 Filter the chloroform through a wad of cotton in a small funnel into a 4-dram vial containing 2 mL of internal standard solution prepared in Step 7.2.4 above.

10.4 Using a hot plate and air jet, start concentrating the first chloroform extract in a hood.

10.5 Continue with the remaining three extracts in Step 10.2.

10.6 Finally, concentrate the chloroform to 0.5 mL and place in a serum cap injector vial (Teflon-lined cap).

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Inject a 1-uL sample into the GC which is set up with the columns and conditions given in Table 1.

13. Calculations

$$\text{Factor} = \frac{(\text{Wt. Component})(\text{Area Internal STD})(1{,}000)}{\text{Area Component}}$$

$$\text{ppm} = \frac{(\text{Factor})(\text{Area Component})}{(\text{Area Internal Standard})}$$

14. Method Performance

14.1 Precision expected is ± 10% relative error.

14.2 The detection limit for each parameter is 0.5 ug/g (ppm).

15. References

15.1 "Alachlor, Propachlor, and Butachlor in Wastewater Streams by GC," Standard Test Method, Monsanto Agricultural Products, St. Louis, MO.

Table 1

Chromatographic Conditions

Injector Temperature	250°C
Detector Temperature	300°C
Column Temperature Program	60°C to 220°C at 10°C/min
Carrier Flow	80 mL/min
Run Time	20 min

Column: 1/4 in x 2 ft 316 Stainless Steel column packed with 10% DCSD 710 on 80/100 Gas Chrom Q.

Note: Columns must be carefully matched or bleed will cause drift problems. Some compensation for bleed can be made by adjusting the carrier flow of the reference columns.

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Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF ALACHLOR, BUTACHLOR, AND PROPACHLOR IN WASTEWATER

METHOD 102

1. Scope and Application

1.1 This method covers the determination of certain acetanilides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Alachlor	77825	15972-60-8
Butachlor	77860	23184-66-9
Propachlor	77729	1918-16-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in waters and treated aqueous effluents at acetanilide herbicide manufacturing facilities.

1.3 The method detection limit (MDL) for each parameter is 0.2 ug/L when using a 1-liter sample.

1.4 The allowable quantities of acetanilides in aqueous waste from herbicide manufacturing sites are limited by both State and Federal environmental protection agencies. In order to monitor acetanilides at anticipated levels of interest, a parts-per-billion (ug/L) method for their quantitation is necessary.

2. Summary of Method

2.1 A volume of water sample (1000 mL) is spiked with an internal standard and then extracted with methylene chloride. The extracts are combined and concentrated to 5 mL. The methylene chloride solvent is exchanged for a toluene/methanol mixture. Analysis for the acetanilides is by packed column chromatography using a nitrogen selective detector routinely and an electron capture detector as necessary to deal with interferences. Component identification is based upon elution times and has been

confirmed by gas chromatographic-infrared and mass spectroscopy techniques.

2.2 If the sample NPD chromatogram contains peaks interfering with alachlor, butachlor, propachlor, or the internal standard, the sample should also be analyzed similarly using an electron capture detector. This change in detector specificity may allow better quantitation.

3. Interferences

3.1 Accurate analyses for parts-per-billion (ug/L) concentrations require rigorous controls to prevent external contamination from the laboratory environment, glassware, reagents, or cross-contamination between samples.

3.2 The laboratory should be maintained at a positive pressure relative to its surroundings, and a suitable air intake filtration system should be used. In addition, to protect the laboratory from contamination carried on the clothing and shoes of manufacturing, formulation, and other laboratory personnel, access to the laboratory where this and other trace analyses are performed should be strictly limited.

3.3 Laboratory Glassware: To avoid contamination from greater than parts-per-billion (ug/L) concentrations of acetanilides, the glassware used for specific parts of the method should be dedicated to just those parts. For example, the equipment for concentrated stock solutions and those for more dilute calibration solutions should not be interchanged.

3.3.1 Glassware cleaning: A rigorous procedure was followed during the development of this method. That procedure was successful since no cross contamination was detected. Since copious volumes of acetone are involved, gloves should be used.

3.3.1.1 Initially glassware is washed with acetone and water until all visible contaminants are removed. Soap is not employed since it too may be a source of interference. The visibly cleaned glassware then is rinsed 10 times with acetone and 30 times by distilled water. Following each 10 water rinses are 5 acetone rinses and finally an extra 5 acetone rinses before the glassware is drained to dryness. Clean glassware is stored in a closed cabinet.

3.3.1.2 Hypodermic syringes are rinsed with acetone immediately after being used. Then, under vacuum, 40 "beads" of water are sucked through the syringe; after each 10 water rinses, 5 acetone rinses are done. Clean syringes are stored in glass test tubes.

3.4 Gas Chromatography: To avoid contamination, the gas chromatograph and analytical columns are dedicated to this analysis.

3.5 Reagents: The best available pesticide grade reagents are employed to assure minimum interferences from this source. It is recommended that solvents from each newly opened reagent container be tested by the analytical procedure to assure that interferences are absent.

3.6 Acetanilide adsorption on glassware: At parts-per-billion (ug/L) levels, tests indicate that certain acetanilides are adsorbed on the surfaces of glassware. A significantly improved recovery can be attained when solutions contain about 20 percent methanol. Similar results were reported for polycyclic aromatics by Ogan, Katz, and Slavin (15.2).

4. Safety

4.1 The solvents needed in this procedure should be used with appropriate precautions and adequate ventilation.

4.2 See the EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

5.1.1 Samples are taken in 1-gallon, amber, glass bottles having a Teflon disk cap insert. Plastic containers cannot be used since components from the container may be dissolved into the sample or sample constituents may be absorbed into the container walls.

5.2 Glassware and Other Equipment

5.2.1 Graduated cylinders, 500 mL and 25 mL.

5.2.2 Separatory funnel, 1000 mL, with Teflon stopcock and stopper.

5.2.3 Evaporative concentrator (modified Kuderna-Danish -- Figure 1).

5.2.4 Disposable glass pipets.

5.2.5 Hypodermic syringe, 10 microliter.

5.2.6 Centrifuge tubes, 5 mL, graduated to 0.1 mL (Fisher Scientific Co., 5-538-35A).

5.2.7 Volumetric flasks, 500 and 1000 mL.

5.2.8 Pipets, 5 and 25 mL.

5.3 Steam bath

5.4 Boiling chips

5.5 Gas Chromatograph: A modern gas chromatograph should be used. It should be equipped for glass columns and on-column injection. In the development of this method, a Hewlett Packard model 5840A gas chromatograph was used.

5.5.1 Analytical column: A glass, 6-ft, 1/4-in O.D., 2 mm-I.D. column packed with 10% OV-11 on Chromosorb W-HP, 100-120 mesh was used.

5.5.2 Gas chromatographic detector: An alkali nitrogen sensitive detector is employed to maximize the sensitivity and selectivity for acetanilides. In the development of this method the Hewlett Packard NPD detector with associated electronics and pneumatics was used.

6. Reagents

6.1 High Grade Distilled in Glass Solvents -- Burdick and Jackson, distilled in glass, pesticide grade

6.1.1 Toluene

6.1.2 Methylene chloride

6.1.3 Methanol

6.2 Acetone -- Fisher Scientific Co., certified ACS grade, A-18

6.3 Analytical Standards

6.3.1 Alachlor

6.3.2 Butachlor

6.3.3 Propachlor

7. Calibration

7.1 GC conditions

The GC analysis is performed isothermally at 200°C for 2 min., followed by programming at 6°C/min to 250°C and holding for 10 min. The operating parameters shown in Table 1 were used on the HP 5840A to provide satisfactory sensitivity, resolution, and quantitation.

7.2 Calibration Procedure

7.2.1 Internal Standard

"Hexoxy Alachlor," analytical standard grade [2-chloro-2′6′-diethyl-N-(hexoxymethyl)-acetanilide], was used.

7.2.2 Calibration and Standardization

The text below describes the preparation of two ranges of standard solutions for use in the two general concentration ranges (0.2-10 ug/L and 20-300 ug/L) for which the method has been validated. The composition of standard solutions used for calibration is as follows for analysis of water and effluents at low levels (0.2 to 10.0 ug/L):

Solution A: Components: Accurately weigh into a 1000-mL volumetric flask 0.02 g of each of the three components to be analyzed for and add methanol to volume. This solution contains 20 ug/mL of each of the materials.

Solution B: Components: Pipet 5 mL of Solution A into a 1000-mL volumetric flask and add methanol to volume. This solution contains 0.1 ug/mL of each of the materials.

Solution C: Internal Standard: Accurately weigh into a 500-mL volumetric flask 0.2 g of "hexoxy alachlor"; add methanol to volume. This solution contains 0.4 mg/mL of the internal standard.

Solution D: Internal Standard: Pipet 5 mL of Solution C into a 1000-mL volumetric flask and add methanol to volume. This solution contains 2 ug/mL of the internal standard.

For waters and effluents where higher levels (20-300 ug/L) of the acetanilide herbicides may be detected and quantitated, standard solutions are prepared such that levels in the calibration samples approximate those occurring in the actual samples. The composition of those standard solutions is as follows:

Solution E: Components: Accurately weigh into a 100-mL volumetric flask 0.02 g of propachlor, 0.20 g of alachlor, and 0.10 g of butachlor. Add methanol to volume. This solution contains 200, 2000, and 1000 ug/mL, respectively, of the three acetanilides.

Solution F: Components: Pipet 5-mL of Solution E into a 100 mL volumetric flask; add methanol to volume. This solution contains 10, 100, and 50 ug/mL, respectively, of the three acetanilides.

Solution G: Internal Standard: Accurately weigh into a 100-mL volumetric flask 0.14 g of hexoxy alachlor; add methanol to volume. This

solution contains 1400 ug/mL of the internal standard.

Solution H: Internal Standard: Pipet 5 mL of Solution G into a 100-mL volumetric flask; add methanol to volume. This solution contains 70 ug/mL of the internal standard.

7.2.3 Instrument and Method Calibration Samples -- To compensate for possible interferences and variable extraction efficiencies, an instrument calibration sample is carried through the same extractive and concentration steps used for a test sample.

7.2.3.1 Measure 500 mL of deionized water into a 1000-mL separatory funnel.

7.2.3.2 To the above, add by pipet 25 mL of Solution B (For low level calibration). I[illegible] alibration at a higher level is necessary, add [illegible] appropriate level of standard solution. The spiking level of the calibration sample should approximate as closely as possible the levels to be [illegible]ured in the actual sample.

7.2.3.3 To [illegible], add by pipet 5 mL of Solution D. (No [illegible] or the higher level range, Solution H would be used.)

7.2.3.4 Add to the above an additional 470 mL of deionized water.

7.2.3.5 Stopper the separatory funnel and mix the contents. This aqueous calibration sample solution contains 2.5 ug/L of each of the components and 10 ug/L of the internal standard. This solution is recommended for use in the analysis of samples of varying composition. During continued analysis of samples of fairly constant composition, a calibration solution more closely reflecting sample composition may produce the best results.

7.2.3.6 Extract the solution prepared in Step 7.2.3.5 with 20 mL of methylene chloride by shaking vigorously for one minute.

7.2.3.7 After the phases separate, draw off the lower methylene chloride layer into the evaporative concentrator.

7.2.3.8 Repeat Steps 7.2.3.6 and 7.2.3.7 three additional times; however, use 7 mL of methylene cloride for each of these extractions.

7.2.3.9 Add a boiling chip (previously extracted in methylene chloride) to the evaporative concentrator; under a stream of nitrogen and with heating by a steam bath, concentrate the volume to approximately 3 mL. This solution must not go dry if accuracy is to be maintained.

7.2.3.10 When the volume is reduced to approximately 3 mL, rinse down the upper flask with approximately 2 mL of toluene and continue heating this solution. The majority of the solvent exchange is indicated by a dramatic decrease in the size of the bubbles. Continue heating approximately 5 minutes to decrease the methylene chloride concentration to a negligible amount. (Solvent exchange is necessary since halogenated solvents cause a marked decrease in the sensitivity of an NPD detector.)

7.2.3.11 Rinse down the walls of the upper flask with approximately 1 mL of methanol.

7.2.3.12 Dry the outside of the ground glass joint, and with a new disposable pipet transfer the concentrate to a 5-mL centrifuge tube.

7.2.3.13 Using no more than 2 mL of toluene, rinse the interior of the lower part of the evaporative concentrator; add these rinses to the 5-mL centrifuge tube.

7.2.3.14 Under a stream of nitrogen, evaporate the solution in the 5-mL centrifuge tube to approximately 0.2 mL.

7.2.3.15 Rinse down the walls of the 5-mL centrifuge tube with 0.1 mL of methanol. Stopper the tube and mix its contents.

7.2.3.16 Inject 3 uL of this solution into the gas chromatograph. Calibrate the instrument; triplicate calibration runs are recommended.

7.2.3.17 The solution from Step 7.2.3.15 is stable for about 5 days. New calibration samples should be prepared weekly. Instrument calibration should be done daily.

8. Quality Control

8.1 Duplicate analysis should be done.

8.2 A 1000-mL distilled water sample, or other suitable blank sample,

is analyzed by this procedure regularly and whenever new containers of solvents are employed.

8.3 See the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Samples are taken in 1-gallon, amber, glass bottles having a Teflon disk cap insert. Plastic containers cannot be used since components from the container may be dissolved into the sample or sample constituents may be absorbed into the container walls.

9.2 Samples should be worked up and analyzed as soon as possible.

9.3 At ug/L levels, rigorous controls are required to prevent inaccurate analyses. Special sample handling is needed to prevent alteration of the sample before analysis and assure that contamination does not occur during the analysis. Protocols should be provided for sample taking, control of the laboratory environment, glassware cleaning, and dedication of instruments and glassware.

9.4 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Measure 1000 mL of aqueous sample into a 1000-mL separatory funnel.

10.2 To the above, add by pipet 5 mL of Solution D. (Note that for the higher level range, Solution H would be used.)

10.3 Stopper the separatory funnel and mix the contents. This aqueous solution contains the sample acetanilides and 10 ug/L of internal standard.

10.4 Extract the acetanilides from the solution prepared in Step 10.4 using methylene chloride. Add 20 mL of methylene chloride and extract by shaking vigorously for one minute.

10.5 After the phases separate, draw off the lower layer into the evaporative concentrator. Do not draw off any emulsion layer that may be at the solvent interface.

10.6 Repeat Steps 10.5 and 10.6 three additional times; however, use 7 mL of methylene chloride for each of these extractions.

10.7 Add a boiling chip (previously extracted in methylene chloride) to the evaporative concentrator; under a stream of nitrogen and with heating by a steam bath, concentrate the volume to approximately 3 mL. This solution must not go dry if accuracy is to be attained.

10.8 When the volume is reduced to approximately 3 mL, rinse down the upper flask with approximately 2 mL of toluene and continue heating this solution. The majority of the solvent exchange is indicated by a dramatic decrease in the size of the bubbles. Continue heating approximately 5 minutes to decrease the methylene chloride concentration to a negligible amount. (Solvent exchange is necessary since halogenated solvents cause a marked decrease in the sensitivity of an NPD detector.)

10.9 Rinse down the walls of the upper flask with approximately 1 mL of methanol.

10.10 Dry the outside of the ground glass joint, and with a new disposable pipet transfer the concentrate to a 5-mL centrifuge tube.

10.11 Using no more than 2 mL of toluene, rinse the interior of the lower part of the evaporator concentrator; add these rinses to the 5-mL centrifuge tube.

10.12 Under a stream of nitrogen, evaporate the solution in the 5-mL centrifuge tube to approximately 0.2 mL.

10.13 Rinse down the walls of the 5-mL centrifuge tube with 0.1 mL of methanol. Stopper the tube and mix its contents. The sample is now ready for gas chromatographic analysis.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Inject 3 uL of the solution from Step 10.13 into the gas chromatograph and determine the amount of each acetanilide present. Table 1 summarizes the recommended operating conditions for the GC.

12.2 If the sample NPD chromatogram contains peaks interfering with propachlor, alachlor, butachlor, or the internal standard, the sample also should be analyzed similarly using an electron capture detector. This change in detector specificity may allow better quantitation.

13. Calculations

13.1 With the Hewlett Packard model 5840A, if the amount of acetanilide in the calibratrion sample is incorporated in units of ug/L, sample results calculated by the microprocessor are reported in ug/L. Other recording/integrating systems may be similarly calibrated for data output.

14. Method Performance

14.1 Range

The methodology has been validated over a range of 0.2-300 ug/L.

14.2 Sensitivity

The minimum level of detection is 0.2 ug/L when using a 1-liter sample.

14.3 Accuracy

In the 0.2-300 ug/L concentration range, recoveries (accuracies) of about 90-110% may be expected. Recoveries are summarized in Table 3.

14.4 Precision

In the concentration range of 0.2 to 10.0 ug/L, the methodology generally may be expected to give precision of ± 20% or better (95% confidence level). In the range of 20 to 300 ug/L, the methodology gives a precision of ± 12% or better (95% confidence level). Table 3 summarizes the pooled coefficients of variation.

15. References

15.1 J.W. Worley, M.L. Rueppel, and F.L. Rupel, "Determination of alpha-Chloroacetanilides in Water by Gas Chromatography and Infrared Spectrometry," Anal. Chem. 52, 1845 (1980).

15.2 K. Ogan, E. Katz, and W. Slavin, "Concentration and Determination of Trace Amounts of Several Polycyclic Aromatic Hydrocarbons in Aqueous Samples," J. Chrom. Sci., 16, 517 (1978).

15.3 F.L. Rupel, J.W. Worley, and R.K. Beasley," Analytical Method for the Determination of Propachlor, Alachlor, and Butachlor in Waters and Effluents at the Parts Per Billion Level" Standard Test Method, Validation Date 3-16-79, Monsanto Agricultural Products Co., St. Louis, Mo.

Table 1

Chromatographic Conditions

Injector Temperature	250°C
NPD Detector Temperature	300°C
Chart Speed	1.00 in/min
Recorder Zero	10.0
Attenuation	2(7)
Slope Sensitivity	0.03
Area Reject	1000
Helium Carrier Flow	25.0 mL/min
Column Temperature	200°C, hold 2 min., then 6°C/min to 250°C, hold 10 min.

Footnote: Column - Glass, 6-ft x 1/4-in O.D., 2-mm ID packed with 10% OV-11 on Chromosorb W-HP, 100-120 mesh.

Table 2

Retention Times

Compound	Retention Time (Minutes)
Propachlor	3.9
Alachlor	7.7
Butachlor	10.6
Hexoxy Alachlor	13.9

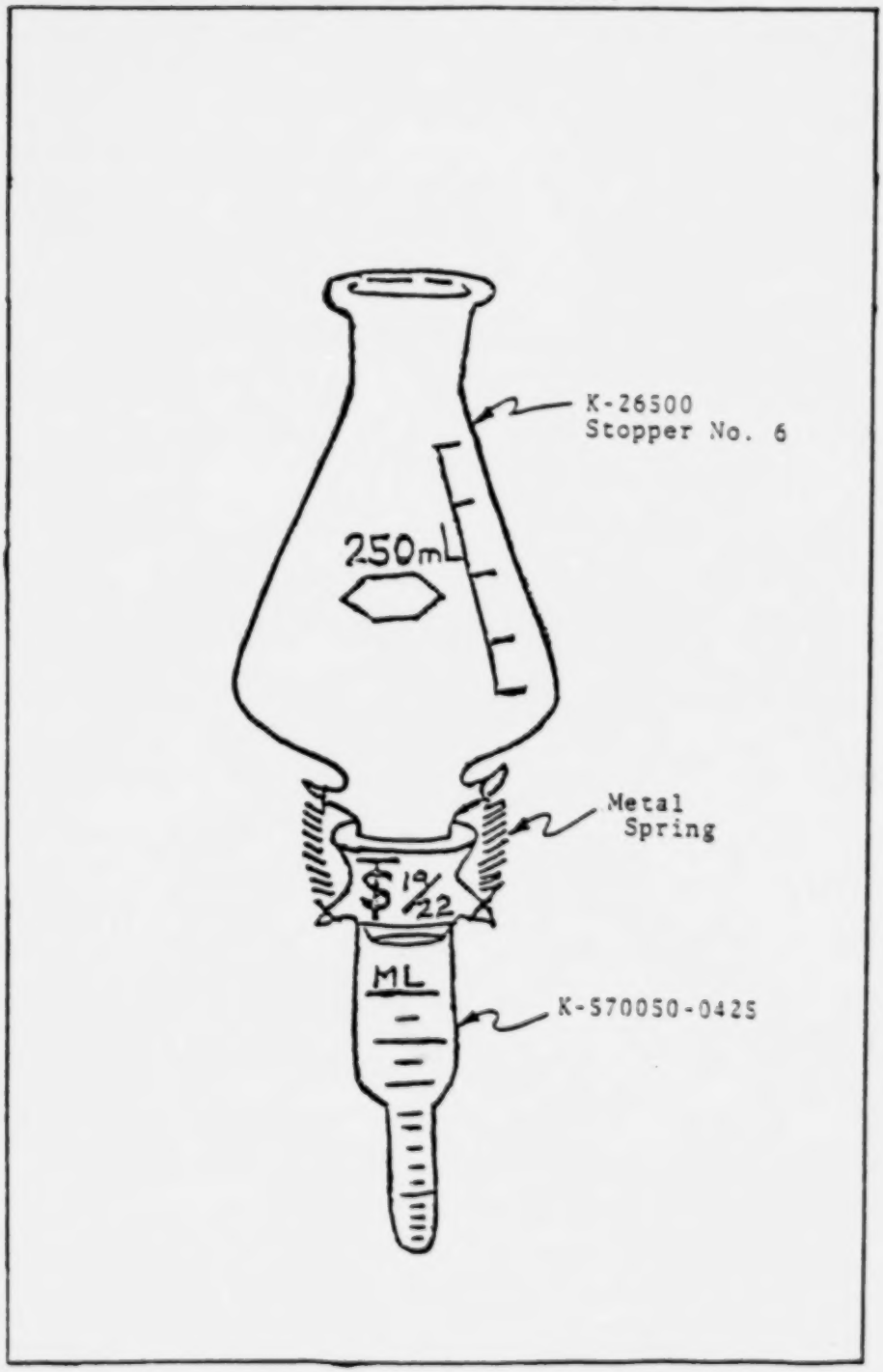
Table 3

Accuracy and Precision

Component	Range, ug/L	Pooled Coeff. of Var.	Recovery, %
Propachlor	0.2-10	0.09	93
	20-30	0.05	96
Alachlor	0.2-10	0.09	94
	200-300	0.06	100
Butachlor	0.2-10	0.10	112
	100-150	0.03	100

Coefficient of Variation = (standard deviation)/(mean).

Figure 1
Attachment 1
Evaporative Concentrator
(Modified Kuderna-Danish)



EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF AOP, ZAC, ZINEB, AND ZIRAM IN WASTEWATER

METHOD 103

1. Scope and Application

1.1 This method covers the determination of certain dithiocarbamates. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
AOP	--	--
Ferbam	--	14484-64-1
Niacide	--	8011-66-3
ZAC	--	--
Zineb	--	142-14-3
Ziram	--	137-30-4

1.2 This method is non-specific for individual dithiocarbamates and results are reported as mg/L of carbon disulfide (CS_2).

1.3 This is a spectrophotometric method applicable to drinking, surface, and saline waters, and domestic and industrial waste effluents.

2. Summary of Method

2.1 A volume of water sample (1-20 mL) is reacted with acid to produce the acid decomposition of the dithiocarbamate followed by the subsequent reaction of the evolved CS_2 with a color reagent, and spectrophotometric determination.

3. Interferences

Not Available.

4. Safety

4.1 Follow the EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Flasks, round-bottom 1000 mL, 3-neck, 28/15, and 35/25 outside balljoints (See Figure 1).

5.2.2 Condenser, water-cooled, 12 inch, with bottom inside 28/15 balljoint and top 5-mm glass tubing extension tipped with an inside 28/15 balljoint (See Figure 1).

5.2.3 Funnel, separatory, graduated, cylindrical, 150 mL with an inside 28/15 balljoint and No. 2 stopcock.

5.2.4 Gas washing bottle, demountable, 150 x 25 mm with 24/40 top joint.

5.2.5 Gas dispersion tubes, with medium fritted cylinders that extend to within 10 mm of bottom of washing bottles. Glass cylinders have one inside and one outside 12/5 balljoint.

5.2.6 Tubing, glass, 5-mm I.D.

5.2.7 Flask, volumetric, with stopper, 25 mL.

5.3 Stirrer, magnetic, "Magnestir"

5.4 Heating mantles, Glas-Col flask heaters, Hemispherical, 1000 mL

5.5 Magnetized stirring bars, Teflon R sealed, 5/16" x 1-1/2"

5.6 Transformers, variable, powerstat

5.7 Spectrophotometer, Beckman Model DB or equivalent

5.8 Tubing, Tygon, 1/4"

6. Reagents

6.1 Hydrochloric acid, 1.25N

6.2 Zinc acetate, 20% aqueous solution

6.3 Nitrogen, compressed gas

6.4 Color Reagent -- To 0.004 g of cupric acetate monohydrate in a 250-mL volumetric flask, add 25 grams of diethanolamine and

dilute to volume with ethanol. This reagent is used for determination of less than 150 ug of carbon disulfide.

7. Calibration

7.1 The optical densities of the standard solutions and standards should be measured using a spectrophotometer operating at a 435-nm wavelength

7.2 Calibration Procedure

7.2.1 Carbon disulfide standard solution. Accurately weigh 1.0 g of carbon disulfide into a 100-mL volumetric flask containing 75 mL of absolute methanol and dilute to volume. Dilute a 1.0-mL aliquot of above solution to 100 mL with absolute methanol. This final solution contains 100 ug/mL of CS_2.

7.2.2 Prepare a standard solution containing 1 ug/mL of CS_2 in methanol. Add, into 25-mL volumetric flasks using a syringe, aliquots of the standard solution equivalent to 25 to 150 ug of carbon disulfide and dilute to volume with a 50/50 v/v mixture of ethanol/color reagent A. Allow the solutions to stand for at least 15 minutes. Measure the optical densities of the solutions against a 50/50 v/v mixture of ethanol/color reagent A using a spectrophotometer operating at a 435-mu wavelength.

8. Quality Control

8.1. Follow the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Digestion

10.1 Assemble the decomposition-absorption apparatus as illustrated in Figure 1. Fill trap I with 15 mL of a 20% aqueous zinc acetate solution. Fill trap II with 12.5 mL of appropriate color reagent.

10.2 Introduce a sample of wastewater through the 35/25 balljoint into the 3-neck round bottom reaction flask. Immediately add 150 mL of boiling 1.25 N hydrochloric acid through the addition funnel. Apply nitrogen pressure and sweep the system at about 200 mL/min. Perform all fortifications just prior to the addition of the acid. Heat the contents to boiling temperature and continue the digestion for 45 minutes.

10.3 Sample size of wastewater depends upon CS_2 concentration. Use 2 mL for 1-2 mg/L; 10 mL for 2-5 mg/L; and 1 mL for 10-15 mg/L.

11. Cleanup and Separation

Not Available.

12. Sample Analysis

12.1 After the digestion period, transfer the contents of trap II to a 25-mL volumetric flask using ethanol as wash liquid. Dilute the contents of the flask to exactly 25 mL with ethanol, stopper, shake, and allow to stand for at least 15 minutes. Measure the absorbance of the sample vs. a 50/50 v/v mixture of ethanol/color reagent spectrophotometrically at 435 mu using 1-cm cuvettes.

13. Calculations

13.1 Plot the optical density readings vs. the concentration of carbon disulfide. Figure 2 represents the standard curve for carbon disulfide concentrations ranging from 25 to 150 ug. The standard curve was linear for solutions containing between 25 and 150 ug of carbon disulfide. Determine the micrograms/25 mL of carbon disulfide using the standard curve:

$$CS_2 \text{ (mg/L)} = \frac{CS_2/25 \text{ mL (from graph)}}{\text{mL wastewater}}$$

14. Method Performance

Not Available.

15. References

15.1 "Spectrophotometric Determination of Dithiocarbamate in Waste Water," Standard Test Method, FMC Corporation, Middleport, NY.

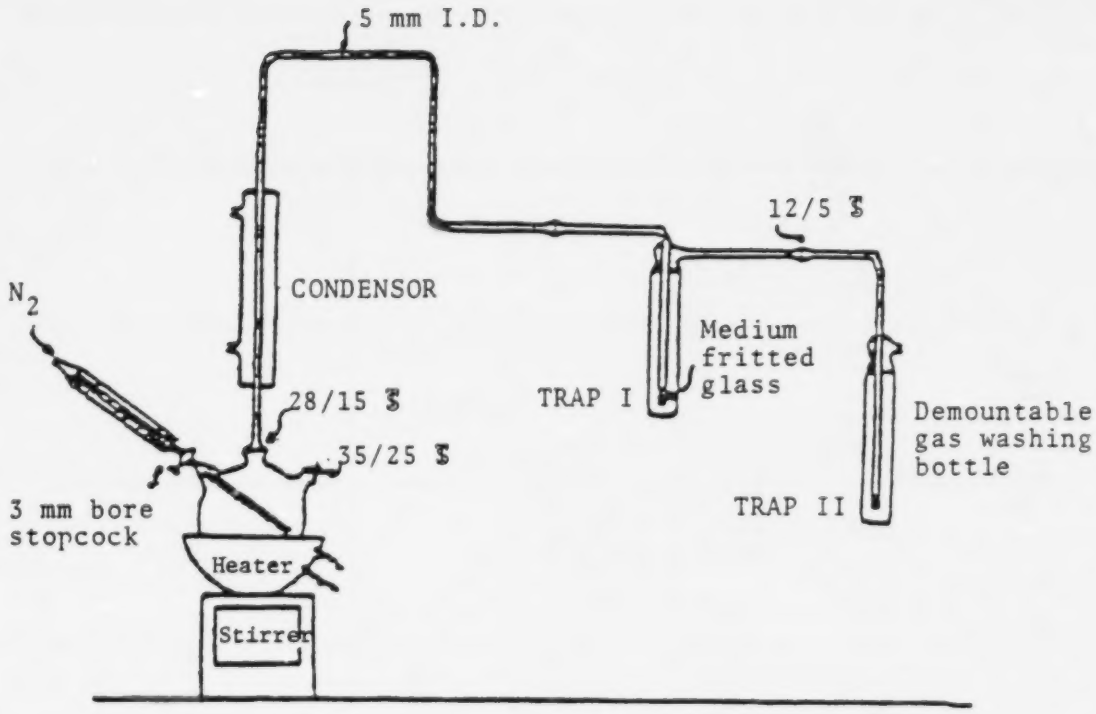
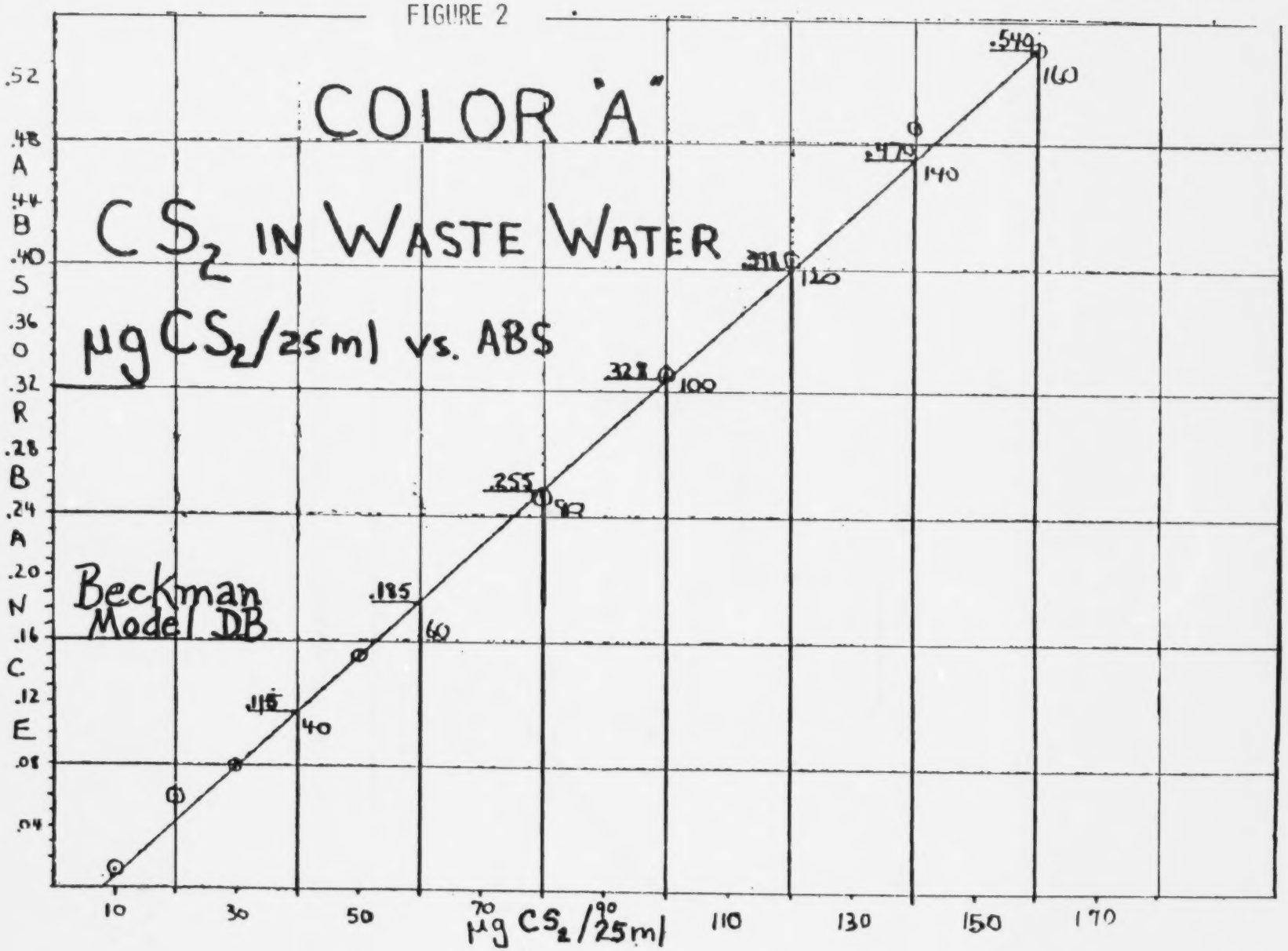


FIGURE I. DECOMPOSITION-ABSORPTION APPARATUS

FIGURE 2


COLOR "A"
CS2 IN WASTE WATER
µg CS2/25ml vs. ABS
Beckman Model DB
ABSORBANCE
.52
.48
.44
.40
.36
.32
.28
.24
.20
.16
.12
.08
.04
10
30
50
70
90
110
130
150
170
µg CS2/25ml
.540
160
.470
140
.398
120
.328
100
.255
80
.185
60
.115
40

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Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BENFLURALIN, ETHALFLURALIN, AND ISOPROPALIN IN WASTEWATER

METHOD 104

1. Scope and Application

1.1 This method covers the determination of certain dinitroanilines. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Benfluralin	39002	1862-40-1
Ethalfluralin	--	55283-68-6
Isopropalin	--	33820-53-0
Trifluralin	39030	1582-09-8

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in water at the part-per-billion level (ug/L), and in water collected at industrial effluent discharge sites in the Wabash River.

1.3 The detection limit is less than 1.0 ug/L for all compounds.

2. Summary of Method

2.1 A measured volume of sample (500 mL) is extracted with chloroform using a separatory funnel. The extract is evaporated to near dryness and the residue is dissolved in benzene. Chromatographic (GC) conditions are established which permit the measurement of the compounds in the benzene extract by electron capture/gas chromatography (GC/EC).

2.2 The method includes a Florisil cleanup procedure to aid in the elimination of interferences.

3. Interferences

3.1 Accurate measurement of these chemicals at the ug/L level requires that laboratory glassware and equipment be kept free

from contamination. The laboratory itself must be located in an area that is secluded from high concentrations of organic compounds. Solvents used in the procedures must be redistilled to remove trace amounts of impurities that can interfere with the detection of the chemicals. The sodium sulfate must also be washed with methanol to remove interfering substances.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not available.

5.2 Glassware and Other Equipment

5.2.1 Redistillation apparatus for redistilling solvents.

5.2.2 Laboratory glassware (pipettes, beakers, flasks, funnels, separatory funnels, and graduated cylinders).

5.2.3 Air-cooled condensing tubes, 24/40 taper, 22-mm O.D.

5.3 Refrigerator or chill room for storage of samples and standard solutions

5.4 Rinco rotary vacuum evaporator with 50°C water bath

5.5 Hot plate with temperature control

5.6 Chromatography columns -- 250-mm x 14-mm I.D. or 300-mm x 11-mm I.D., equipped with stopcocks

5.7 Gas-liquid chromatograph equipped with electron capture detector

5.8 Gas-liquid chromatograph equipped with flame photometric detector (optional)

6. Reagents

6.1 Solvents -- reagent grade

6.1.1 Chloroform (redistilled)

6.1.2 Benzene (redistilled and stored over 5A molecular sieves -- McFaul Co., Cincinnati, OH)

6.1.3 Hexane (redistilled)

6.1.4 Carbon tetrachloride (redistilled)

6.1.5 Methanol

6.1.6 Pyridine

6.2 1% Isopropanol in acetonitrile

6.3 Concentrated HCl: methanol (1:1)

6.4 5% Sodium chloride solution

6.5 Methyl iodide (Matheson, Coleman, & Bell, 98% Stabilized Industrial)

6.6 Sodium carbonate, anhydrous

6.7 Sodium sulfate, anhydrous, methanol washed

6.7.1 Washing Procedure for Sodium Sulfate -- Connect a 2-liter separatory funnel to a large vacuum filtering flask fitted with a rubber stopper. Fill the flask 4/5 full with sodium sulfate, and wash the sodium sulfate with a total of about 2 liters of methanol while pulling a vacuum. The sodium sulfate can then be removed from the funnel and air dried or dried by pulling a vacuum on a large Buchner funnel.

6.8 Phosphorous tribromide (practical)

6.9 5% Sodium hydroxide solution

6.10 Alumina, Alcoa F-20, deactivated with 4% water and sieved to 120/170 mesh

6.11 Florisil, 100/120 mesh, deactivated with 8% water

6.12 Trifluoroacetic anhydride (redistilled)

6.13 1.25N NaOH

6.14 Carborundum boiling chips

6.15 1.25N HCl

7. <u>Calibration</u>

7.1 Analyze the compounds by gas chromatography using electron capture detection under the conditions given in Table 1

7.2 Calibration Procedure

7.2.1 Direct standard concentration -- 0.05 ug/mL in benzene

7.2.2 Note: Direct standards for trifluralin and benfluralin should be injected prior to each set of samples to ensure

that these two compounds can be distinguished by their retention times.

8. Quality Control

8.1 Control samples and standard recovery samples should be carried through the assay procedures for each set of experimental samples if results are desired with an accuracy of about ± 10%. If only approximate values (± 25-30%) are acceptable, the control and recovery samples can be omitted.

8.2 Assay a control sample and a recovery sample in exactly the same manner as experimental samples. Use tap water for the control sample and prepare a spiked recovery sample at a concentration of 0.001 mg/L by fortifying 500 mL of control tap water with 0.50 ug of each compound being assayed.

8.3 See the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Transfer 500 mL of water to a 1-liter separatory funnel; add 50 mL of chloroform, shake for 30 seconds, and allow the phases to separate.

10.2 Eliminate persistent emulsions by draining the lower chloroform layer through a funnel containing sodium sulfate into a 250-mL flat-bottom flask.

10.3 Repeat the extraction with two additional 50-mL portions of chloroform and wash the sodium sulfate with 10 mL of chloroform.

10.4 Evaporate the chloroform just to dryness with a Rinco evaporator and immediately dissolve the residue in 50.0 mL of benzene.

10.5 Transfer a 10.0-mL aliquot (Solution A) of the benzene to a 125-mL flat-bottom flask.

10.6 The evaporating flasks must be removed from the Rinco immediately after the chloroform has evaporated to prevent the loss of volatile compounds such as trifluralin.

11. Cleanup and Separation

11.1 Evaporate Solution A just to dryness and dissolve the residue in 5 mL of hexane.

11.2 Follow the Florisil column procedure in Steps B-8 through B-14 of Procedure 5801616 (Eli Lilly reference method, not enclosed herein) with the following modification: In Step 12, add 120 mL

of hexane in order to elute ethalfluralin, isopropalin, and benfluralin in addition to trifluralin.

Note: The Florisil must be deactivated and standardized prior to initial use as described in Section D of Procedure 5801616 (Eli Lilly reference method, not enclosed herein). The Florisil must be standardized to include isopropalin, as well as trifluralin.

11.3 Evaporate the sample just to dryness and dissolve the residue in 2.0 mL of benzene.

2. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 The electron capture detector has only a limited range in which the peak response is linear with concentration. Therefore, the experimental samples must be diluted to yield a response that is similar in magnitude to that of the direct standard. Likewise, samples analyzed by flame photometric detection may require dilutions to yield a peak response that falls within the range of the standard curve. The magnitude of the dilutions must then be accounted for in the dilution factor (DF) of the calculations.

13. Calculations

13.1 Percent recovery in standard recovery sample:

$$\% \text{ Recovery} = \frac{\text{PR (recovery sample)} - \text{PR (control)}}{\text{PR (direct standard)}} \times 100$$

where PR = peak response

13.2 Parts-per-million (mg/L) residue in water sample:

$$\text{ppm} = \frac{(\text{ug/mL}) \times \text{DF} \times \text{AF} \times 100}{\text{SV} \times \% \text{ Recovery}}$$

$$\text{where ug/mL} = \frac{\text{PR (sample)}}{\text{PR (standard)}} \times 0.05 \text{ ug/mL}$$

DF = dilution factor (2.0 mL, unless sample is further diluted for analysis)

AF = aliquot factor (AF=5, since each aliquot was 1/5 of the extract)

SV = sample volume (500 mL)

14. Method Performance

Not Available.

15. References

15.1 "Determination of Trifluralin and Other Elanco Herbicides in Water," Procedure 5801665, Eli Lilly and Company, Lilly Research Laboratories, Greenfield, IN.

Table 1

Chromatographic Conditions

Temperature - Oven	120°C
- Flash heater	210°C
- Detector	290°C
Carrier gas	Argon/methane (90:10), flow rate - 50 mL/min
Electrometer - Range	1
- Attenuation	32
Injection volume	3.0-5.0 microliters
Column	122-cm x 3-mm I.D. glass packed with "Aue" Carbowax 20M

Note: The "Aue" packing was specially prepared by Eli Lilly personnel.

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TEST METHOD

DETERMINATION OF BENOMYL AND CARBENDAZIM IN WASTEWATER

Method 105

1. Scope and Application

1.1 This method covers the determination of benomyl and carbendazim.

Parameter	Storet No.	CAS No.
Benomyl	--	17804-35-2
Carbendazim	--	10605-21-7

1.2 Benomyl cannot be determined directly by this method. Both compounds are measured and reported as carbendazim.

1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in aqueous waste effluents.

1.4 The method detection limit (MDL) is 0.08 ug/g (ppm) carbendazim.

2. Summary of Method

2.1 A measured volume of sample (100 mL) is acidified to hydrolyze benomyl to carbendazim. The total carbendazim is extracted with ethyl acetate. Determination is by high-speed cation exchange liquid chromatography.

3. Interferences

Not Available.

4. Safety

4.1 Methanol is a colorless liquid with a faint alcoholic odor. The threshold limit value (TLV) is 200 ppm, according to the American Conference of Governmental Industrial Hygienists (ACGIH). This

material is flammable and toxic, if ingested, causing blindness or death. The odor threshold is 100 ppm.

4.2 Ethyl acetate is a flammable liquid with a TLV of 400 ppm (ACGIH).

4.3 Sodium hydroxide is a strongly alkaline, corrosive solid with a TLV of 2 mg/m^3 (ACGIH). The minimum protection of Butal-sol rubber gloves, rubber sleeves, a rubber apron, and a face shield should be worn. In case of contact, flush with large quantities of water.

4.4 Benomyl is a stable, light tan powder with moderate toxicity having a TLV of 10 mg/m^3(ACGIH).

4.5 Acetone is a colorless, highly flammable liquid which has a sweet pungent odor. Its vapors form explosive mixtures; its TLV is 750 ppm (ACGIH).

4.6 Hydrochloric acid is a colorless, fuming liquid with a pungent odor. It is highly corrosive to body tissue. Its TLV is 5 ppm (ACGIH). Vapors may cause severe burns to the upper respiratory tract and eyes. Butal-sol rubber golves, rubber sleeves, a face shield, and rubber apron should be worn.

4.7 Carbendazim is a fungicide with very low oral toxicity (LD_{50} for rats, oral, is >11,000 mg/kg).

4.8 Ammonium hydroxide is a colorless liquid with a TLV of 25 ppm (ACGIH). It is strongly alkaline by nature. The minimum protective equipment which should be worn is listed above.

4.9 Phosphoric acid is a viscous, light-colored liquid with a TLV of 1 mg/m^3 (ACGIH). It is a corrosive material and should be treated with caution.

4.10 n-Hexane is a colorless, volatile liquid with a faint, peculiar odor. Handle in a hood and avoid breathing the vapors. It is flammable.

4.11 Consult safety sheets and handbooks for further information regarding these materials.

4.12 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Evaporative concentrators, Cat. No. K659300, Kontes, Vineland, NJ.

5.2.2 Micropipets, disposable "9 in", Cat. No. P5200-2, Scientific Products, Obetz, OH.

5.2.3 Syringe, 1-mL B-D Yale Luer-Lok[tm] tip tuberculin, Cat. No. 14-820-16, Fisher Scientific, King of Prussia, PA 19406.

5.3 Fluoropore filters, 0.5 um, Cat. No. FHLP01300, Millipore Corp., Bedford, MA 01730

5.4 Filter assembly, Swinney, SS, 13 mm, Cat. No. 3001200, Millipore Corp., Bedford, MA 01730

5.5 Guard column kit, CXHC Pellionex, SCX Guard Column kit, Whatman, Inc., Clifton, NJ 07014

5.6 Tube heater, standard model, Cat. No. K720000, Kontes, Vineland, NJ

5.7 Hewlett-Packard Model 1084B, liquid chromatograph, equipped with auto sampler and variable wavelength UV detector, Hewlett-Packard Co., Avondale, PA 19311

5.7.1 Partisil SCX LC column, PXS-1025, Cat. No. 4227-104, Anspec Company, Ann Arbor, MI 48107

6. Reagents

6.1 Ethyl acetate, distilled-in-glass, pesticide grade, Burdick and Jackson Labs, Muskegan, MI

6.2 n-Hexane, distilled-in-glass (UV), Burdick and Jackson Labs, Muskegan, MI

6.3 Methyl alcohol, distilled-in-glass (UV), liquid chromatography quality, Fisher Scientific, Parkersburg, WV

6.4 Ammonium hydroxide, concentrated

6.5 Phosphoric acid, 85%, MCB

6.6 Hydrochloric acid, concentrated

6.7 Sodium hydroxide, 50%, MCB

6.8 Benomyl standard, Experimental Station, Dupont, Wilmington, DE

6.9 Carbendazim standard, Experimental Station, Dupont, Wilmington, DE

6.10 2-Aminobenzimidazole standard, Experimental Station, Dupont, Wilmington, DE

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those in Table 1

7.2 Calibration Procedure

7.2.1 Weigh approximately 0.5 g (± 0.0001) of methyl-2-benzimidazole carbamate (MBC) into a clean, dry 1000-mL volumetric flask. Add 100 mL of methanol to dissolve and dilute to the mark with 0.1 N H_3PO_4 (Step 7.2.4). Mix well. This prepares a 500-ug/mL MBC standard.

7.2.2 Pipet 10.00 mL of the standard stock solution prepared above into a clean, dry, 100-mL volumetric flask and dilute to the mark with 0.1 N H_3PO_4. This represents a 50-ug/mL MBC standard (Step 7.2.3).

7.2.3 Depending on the sample concentration, further dilutions may be necessary. The standard concentration should be matched with the sample concentration.

7.2.4 Prepare 0.1 N H_3PO_4 by diluting 5.00 mL of 85% H_3PO_4 to 2000.0 mL with liquid chromatographic grade water.

8. Quality Control

8.1 Follow the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Tare weigh a 250-mL beaker on a top-loading pan balance.

10.2 Add approximately 100 mL of the well-mixed wastewater sample to the beaker and weigh to the nearest 0.01 g. Record the weight. The sample should be adjusted to pH 4-5 with sodium hydroxide or acid prior to preparation for extraction.

10.3 Add 2 mL of concentrated hydrochloric acid to the sample contained in the 250-mL beakers, verify the pH with pH test paper (should be pH = 2, if not, add more acid), cover with a watch glass and heat on a 90°C hot plate or steam bath for 30 minutes.

10.4 Using an ice bath, cool and then transfer the sample to a 500-mL separatory funnel with the aid of several small deionized water washings.

10.5 Employing the 250-mL beaker in which the sample was heated, add 100 mL of pesticide grade n-hexane to the separatory funnel. Shake for 2 minutes, allow the phases to separate, collect the aqueous phase (lower) in the 250-mL beaker and discard the hexane layer (top).

10.6 Repeat step 10.5 above.

10.7 Add 20 mL of 6.5 N NaOH to the aqueous layer, verify the pH (should be approximately pH = 10, if not basic, add more caustic), and extract immediately with four 100-mL portions of pesticide quality ethyl acetate. Use 2-minute shaking periods for each extraction. Use the 250-mL beaker as before (Step 10.5).

10.8 Allow the phases to separate and filter the 100-mL portions of ethyl acetate (top layers) through about 50 g of anhydrous sodium sulfate into a 500-mL round-bottomed flask.

10.9 For each sample, concentrate the combined ethyl acetate extracts to about 10 mL using a rotary vacuum evaporator with a bath temperature of 60°C.

10.10 Quantitatively transfer the concentrated extracts to a 12-mL Kontes evaporator tube using a 9-inch pasteur pipette and several small volumes of ethyl acetate as wash.

10.11 Using a Kontes tube heater with a nitrogen bath over the samples, further concentrate the extract to about 2 mL. Add 1 mL of 1.0 N H_3PO_4 and continue to evaporate the remaining ethyl acetate.

10.12 Quantitatively transfer the aqueous solution remaining to a 2-mL volumetric flask using a pasteur pipette and washes of 0.1 N H_3PO_4.

10.13 Dilute to volume with 0.1 N H_3PO_4 and mix thoroughly.

10.14 Filter each sample solution through a 0.5-um millipore filter, using a syringe and Swinney adaptor, into the 2-mL glass vial. Cap and label the vial.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Dilute 1.00 mL of concentrated ammonium hydroxide to 2000.0 mL in a volumetric flask. Adjust the pH to 1.7 (± 0.04) using a pH meter and concentrated phosphoric acid. Filter through 0.5-um millipore filter paper.

12.2 Equilibrate the liquid chromatograph under the conditions shown in Table 1.

12.3 Inject standard and samples and allow chromatograms to develop. Under these conditions the uncorrected retention times of MBC and 2AB (Step 12.4) are approximately 8 and 12 minutes, respectively.

12.4 2-Aminobenzimidazole (2AB) may be employed in the standard as a method for assessing the column performance. 2AB may be prepared in the same manner as MBC (Step 7.2.1) and both diluted to 100 mL in the same volumetric flask (Step 7.2.2).

13. Calculations

13.1 Measure and record the peak height (mm) of MBC for each sample and standard.

13.2 Calculate and report the concentration of MBC found in ug/g as follows:

$$\text{ug/g MBC} = \frac{(CF)\ (PHs)\ (AF)}{SW}$$

Where:

$$CF = \text{calibration factor} = \frac{[MBC]\ \text{standard (ug/mL)}}{\text{peak height Std. (mm)}}$$

This should be an average of all standards analyzed, corrected to the same attenuation.

PH_s = peak height of MBC in sample (mm) corrected to attenuation for which standard was analyzed.

AF = aliquot factor = final volume to which sample was diluted after extraction (mL)

SW = weight of sample (g)

14. Method Performance

14.1 Based on non-consecutive replicate analyses, the precision is approximately 2% relative at the 50-ug/g MBC level. Average recovery for MBC was 88% at the 0.4-ug/g level. Using a 100-g sample the theoretical sensitivity is 0.08 ug/g MBC.

15. References

15.1 American Conference of Governmental Industrial Hygienists, "Documentation of the Threshold Limit Values for Substances," 1979.

15.2 "Determination of Benomyl/Carbendazim, Liquid Chromatographic Technique," Method No. W28.047(R), 4/14/81, E.I. duPont de Nemours and Co. (Inc.) HQ, TX.

Table 1

Chromatographic Conditions

Solvent temperature B (Methanol)	35°C
Solvent temperature A (Buffer)	60°C
Flow	3 mL/min.
% B (Methanol)	60
% A (0.007 N $NH_4H_2PO_4$ buffer)	40
Maximum pressure	400 bar
Minimum pressure	1 bar
Oven temperature	40°C
Wavelength sample: Reference	274 nm:0 nm
Chart speed	0.5 cm/min.
Zero	5
Area Rej.	0
Slope sensitivity	0.3
Attenuation	2 8 (AU x 10^{-4})
Injection volume	50 uL

Column: Partisil SCX LC column, PXS-1025, Cat. No. 4227-104, Anspec Company, Ann Arbor, MI 48107

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TEST METHOD

DETERMINATION OF BENOMYL AND CARBENDAZIM IN WASTEWATER

Method 106

1. Scope and Application

1.1 This method covers the determination of benomyl and carbendazim.

Parameter	Storet No.	CAS No.
Benomyl	--	17804-35-2
Carbendazim	--	10605-21-7

1.2 Benomyl cannot be determined directly by this method. Benomyl is hydrolized to carbendazim, and both compounds are measured and reported as carbendazim.

1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in aqueous waste effluent.

1.4 The method detection limit (MDL) is 0.01 ug/g (ppm).

2. Summary of Method

2.1 An aliquot of sample (100 g) is acid hydrolized, extracted with ethyl acetate, and subsequently analyzed by high speed cation exchange liquid chromatography.

3. Interferences

Not Available.

4. Safety

4.1 Follow the EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not available.

5.2 Glassware and Other Equipment

5.2.1 Beakers, 250 mL

5.2.2 Separatory Funnel, 500 mL

5.2.3 Fine tip dropper

5.3 Steam bath

5.4 Rotary Evaporator

5.5 Liquid Chromatograph

6. Reagents

6.1 Concentrated Hydrochloric Acid

6.2 n-Hexane

6.3 Ethyl Acetate

6.4 6.5 N NaOH

6.5 .1 N Phosphoric Acid

6.6 Benomyl Standard

6.7 Carbendazim Standard

7. Calibration

7.1 Chromatographic Conditions are given in Reference 15.1.

7.2 Calibration Procedure -- Measure the peak height for MBC and determine the micrograms of this compound in the aliquot using a previously prepared calibration curve (Reference 15.1).

8. Quality Control

8.1 Follow the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Weigh a 100-g sample into a 250-mL beaker, add 2 mL of concentrated HCl, cover, and heat on a steam bath for about 30 minutes.

10.2 Cool and transfer to a 500-mL separatory funnel using several small water washes. Add 100 mL n-hexane, shake for 2 minutes, and allow the phases to separate. Discard the hexane layer. Repeat the hexane wash using a second 100-mL portion of n-hexane.

10.3 Make the aqueous phase strongly basic by adding 15 mL of 6.5 N NaOH and extract immediately with four 100-mL portions of ethyl acetate using 2-minute shaking periods for each extraction.

10.4 Allow the phases to separate and filter the ethyl acetate phase through anhydrous sodium sulfate into a 500-mL round-bottomed flask.

10.5 Concentrate the combined ethyl acetate extracts to about 10 mL using a vacuum rotary evaporator at 60°C.

10.6 Quantitatively transfer the concentrated extract to a 30-mL beaker using several small volumes of ethyl acetate as wash. Continue to concentrate the solvent to about 3-5 mL. Add 1 mL of 0.1 N H_3PO_4 and continue the evaporation until all of the ethyl acetate is removed.

10.7 Quantitatively transfer the aqueous solution to a 2-mL volumetric flask using a fine tip dropper and several small washes of 0.1 N H_3PO_4. Dilute to volume with 0.1 N H_3PO_4 and mix thoroughly.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Equilibrate the liquid chromatograph and inject an aliquot of the prepared extract as described in the reference method (Reference 15.1).

13. Calculations

13.1 Calculate the amount of carbendazim present in ug/g (ppm) by dividing the micrograms of MBC found, corrected for aliquot factor, by the sample weight in grams. To express the residue as ppm benomyl, divide the micrograms of MBC found, corrected for the molecular weight conversion (1.53) and aliquot factors, by the sample weight in grams.

14. Method Performance

14.1 Using a 100-g sample, the method has a sensitivity of 0.01 ug/g (ppm).

15. References

15.1 Kirkland, J.J., Holt, R.F., Pease, H.L. Determination of Benomyl Residues in Soils and Plant Tissues by High-Speed Cation Exchange Liquid Chromatography, J. Agr. Food Chem., 21, 368 (1973).

15.2 Pease, H.L., Gardiner, J.A. Fluorometric and Colorimetric Procedures for Determining Residues of Benomyl, J. Agr. Food Chem., 17, 267 (1969).

15.3 "Determination of Benomyl/Carbendazim in Plant Aqueous Waste Effluent," Attachment 3, E.I. duPont de Nemours and Company, Belle, WV Plant.

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TEST METHOD

DETERMINATION OF BENTAZON (BAS 351-H) IN WASTEWATER

METHOD 107

1. Scope and Application

1.1 This method covers the determination of bentazon (BAS-351-H).

Parameter	Storet No.	CAS No.
Bentazon (BAS 351-H)	--	25057-89-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

1.3 The method detection limit (MDL) is 10 ug/L.

2. Summary of Method

2.1 An aliquot of sample (100g) is acidified and extracted with ethyl acetate. The extract is dried, concentrated to 1-2 mL, and methylated with diazomethane. The methylated extracts are analyzed by gas chromatography with flame photometric detection.

3. Interferences

Not Available.

4. Safety

4.1 Follow the EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnels, 500 mL, Kimax or equivalent.

5.2.2 Graduated concentrator tubes, 35 mL, Kimble, 24/40 stoppers.

5.2.3 Flash Evaporator, Buchi or equivalent.

5.3 Diazomethane Generator, available from Aldrich Chemical Company

5.4 Gas Chromatograph, Tracor-Microtek, Model MT 220 equipped with a Flame Photometric detector

5.4.1 Gas Chromatographic Column:

5.4.1.1 10% SE-30 on Chromosorb G, HP or Gas Chrom Q, 80/100 mesh 4-ft x 1/4-in I.D.

5.4.1.2 3% Carbowax 20M on Gas Chrom Q, 60/80 mesh, 4-ft x 1/4-in I.D.

6. Reagents

6.1 Ethyl Acetate, MCB, pesticide quality

6.2 Sodium Chloride, Baker reagent

6.3 Sodium Sulfate, Baker anhydrous reagent (prewash with chloroform to remove extraneous sulfur peaks)

6.4 Ethyl Ether, absolute, pesticide quality

6.5 Diazomethane solution: Prepare from Diazald reagent as specified by the manufacturer, Aldrich Chemical Co.

6.6 Hydrochloric Acid, 12 N, reagent

6.7 Acetone, pesticide quality, MCB

6.8 Bentazon (BAS 351-H), Analytical standard, BASF Wyandotte Corporation

7. Calibration

7.1 Sample residues are dissolved in acetone or benzene and injected into a gas chromatograph equipped with a Flame Photometric detector operating in the sulfur-specific mode (394-nm filter), operating at the conditions given in Table 1 for methylated Bentazon (BAS 351-H). Gas chromatographic separations are made using a 10% SE-30 or 3% Carbowax 20M column on Gas Chrom Q or Chromosorb G packing.

7.2 Calibration Procedure

7.2.1 Prepare a stock standard solution by weighing 100.0 mg of Bentazon (BAS 351-H) analytical standard, and quantitatively transfer to a 100-mL volumetric flask. Dilute the material to 100 mL with acetone. This solution contains 1 ug/uL (stock solution A).

7.2.2 Transfer 10.0 mL of this stock solution A to a 35-mL graduated concentrator tube and evaporate the acetone to dryness.

7.2.3 Dissolve the Bentazon (BAS 351-H) residue in 10-mL ethyl ether and add 20 mL diazomethane solution. Allow the solutions to react for 10 minutes.

7.2.4 Concentrate the solvent to approximately 2 mL on an N-EVAP.

7.2.5 Quantitatively transfer the methylated Bentazon (BAS 351-H) solution to a 10-mL volumetric flask. Wash the 35-mL concentrator tube with acetone and add this to the 10-mL volumetric flask. Adjust the volume to 10 mL with acetone. This is stock solution B containing 1 ug/uL of methylated Bentazon (BAS 351-H) standard.

7.2.6 Stock solution B may be used for preparing injection standards by serial dilutions of microliter quantities.

7.2.7 A series of dilutions of methylated Bentazon (BAS 351-H) (stock solution B) containing 0.5, 1, and 2 ng/uL are made. Inject 2-10 uL of each diluted standard solution to obtain a standard curve for 1.0 to 10.0 ng standards. Higher ranges may be used if so desired.

7.2.8 Determine the peak height or area of each standard injection.

7.2.9 Construct a standard curve by plotting detector response (peak height or area) versus concentration (ng or ug) of standard methylated Bentazon (BAS 351-H) injected. A typical standard curve for the flame photometric detector is shown in Figure 1. Note: Since the response of the flame photometric detector is not linear, standard curves may be plotted on log-log paper to obtain a straight line curve.

7.2.10 Bracket every 3-5 samples analyzed with known amounts of standard to maintain a continual check for any shifts in sensitivity.

8. <u>Quality Control</u>

8.1 Follow the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 If samples contain no sediment, go to Step 10.2; however, if sediment is present, shake the samples well to get a homogenous sample aliquot.

10.2 Measure out 100 mL equivalent to 100 g of sample into a 500-mL separatory funnel.

10.3 Dilute the sample to 250 mL by adding sodium chloride solution (150 g in 2 L distilled water).

10.4 Make the resultant solution acidic (pH <1) by adding concentrated hydrochloric acid dropwise.

10.5 Extract the acidified solution 3 times with 50 mL ethyl acetate.

10.6 Dry the extracts through a pad of sodium sulfate which has been prewashed with chloroform to remove extraneous sulfur peaks, and collect them in a 250-mL Erlenmeyer flask.

10.7 Concentrate the combined extracts to 1-2 mL.

10.8 Take the residue from 10.7 and add 10 mL of ethyl ether and 1 mL of diazomethane solution.

10.9 Allow the mixture to react for 10 minutes at room temperature.

10.10 Quantitatively transfer the methylated residues to a 35-mL graduated concentrator tube with repeated acetone washings.

10.11 Concentrate the solution to approximately 1 mL.

10.12 Adjust the volume to an appropriate level with acetone for final determination by gas chromatography.

11. Cleanup and Separation

Not available.

12. Gas Chromatography

12.1 Inject an appropriate aliquot into the gas chromatograph and determine the peak height or area for methylated Bentazon (BAS 351-H).

12.2 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

13. Calculations

13.1 Directly compare the peak heights or areas of unknown samples injected with the appropriate standard curve and extrapolate the concentration of methylated Bentazon (BAS 351-H) in the injected sample aliquot.

13.2 Determine the residue results in terms of ppm methylated BAS 351-H in the sample by the following:

$$\text{ppm} = \frac{\text{ng Found}^{a}}{\text{mg sample injected}}{}^{b} \text{ divided by (R), where}$$

a = concentration extrapolated from standard curve

$$b = \frac{\text{mg aliquot}}{\text{for analysis}} \times \frac{\text{microliters injected}}{\text{microliters final volume}}$$

R = Recovery factor based on fortified controls carried through the procedure. R is expressed as a decimal (i.e. 100% = 1.00, 90% = 0.90, etc.)

14. Method Performance

14.1 Recoveries for water are tabulated in Table 2. They range from 84 to 116% in samples from Texas and Mississippi, involving four field experiments.

15. References

15.1 LOTEL Report No. 133, October 2, 1973, "Determination of BAS 351-H Residues in Soil and Runoff Water," James Devine, Lake Ontario Environmental Laboratory, Oswego, New York.

15.2 Lab Report dated April 1972, "Method of Analysis for the Determination of Bentazon in Drinking and Leaching Water," BASF Analytical Laboratories, Limburgerhof, Germany.

15.3 "Determination of BAS 351-H [3-isopropyl-1H-2.1.3-benzothiadiazin-4(3H)-one-2,2-dioxide] Residues in Water by Gas Chromatography," Analytical Method No. 8, January 23, 1974, BASF Wyandotte Corporation, Geismar, LA.

Table 1

Gas Chromatographic Conditions for Flame Photometric Detection of Methylated Bentazon (BAS 351-H) Residues in Water

Gas Chromatograph	Tracor-Microtek MT 220 equipped with a Flame Photometric detector operating in the sulfur specific mode (394-nm filter)
Column	a. 3% Carbowax 20M on Gas Chrom Q, 60/80 mesh, 6-ft x 1/4-in I.D.
	b. 10% SE-30 on Chromosorb G, H.P. or Gas Crom Q, 80/100 mesh, 4-ft x 1/4-in I.D.
Temperatures:	
Glass Inlet	225°C
Detector	200°C
Column	200°C
Gas Flows:	
Carrier	Nitrogen 80 mL/min
Hydrogen	60 mL/min
Oxygen	20 mL/min
Air	15 mL/min
Electrometer:	
Sensitivity	$-X^2(10^{-8})$
Attenuation	10^2 x 64
Recorder:	
Range	1 mV
Chart Speed	0.25 inches/minute
Minimum Detection Limit	1 ng Methylated Bentazon (BAS 351-H)-
Retention Time (Approx)	3.0 - 3.5 min

Table 2

Typical Recoveries of Methylated Bentazon (BAS 351-H) Residues in Water

Sample Matrix	Fortification Level (ug/g) Bentazon (BAS 351-H) Added	% Recovery
Water	0.50	95
	0.10	105
	0.10	94
	0.10	90
	0.10	104
	0.05	84
	0.01	94
	0.01	94
	0.01	116
	0.01	103
	0.01	108
	Average =	98.8%

FIGURE 1

TYPICAL STANDARD CURVE OF
METHYLATED BAS 351-H

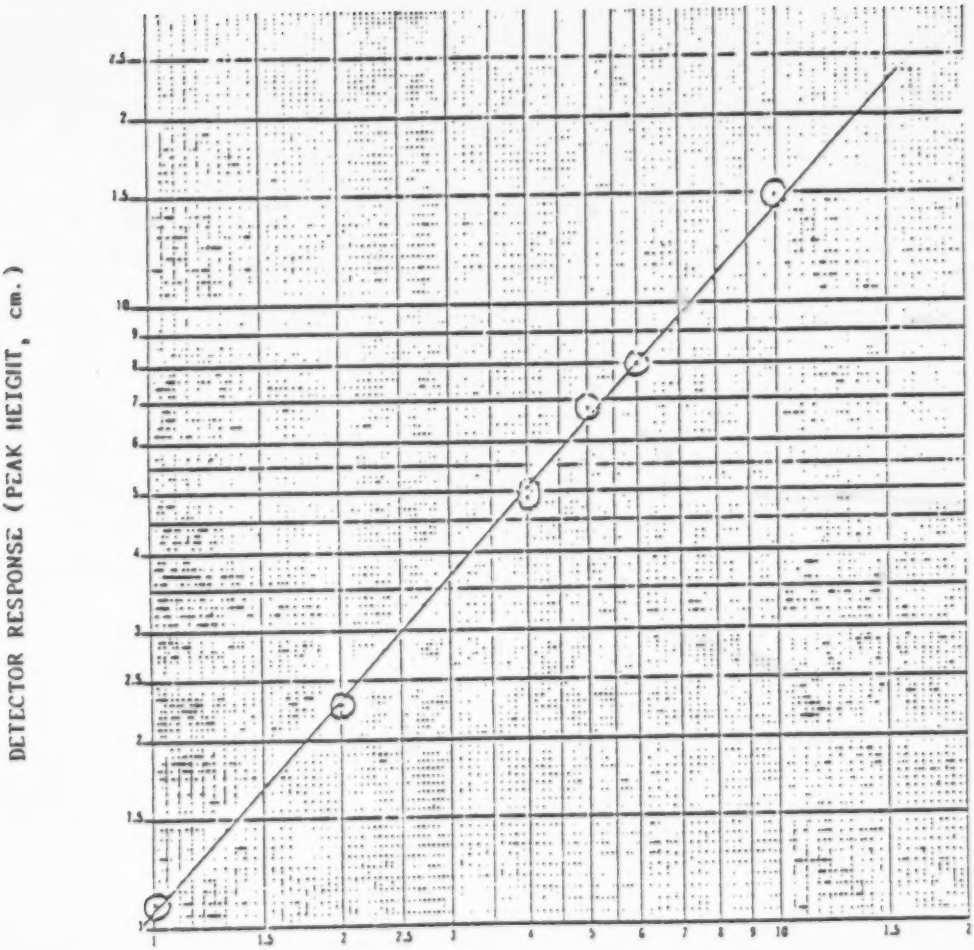
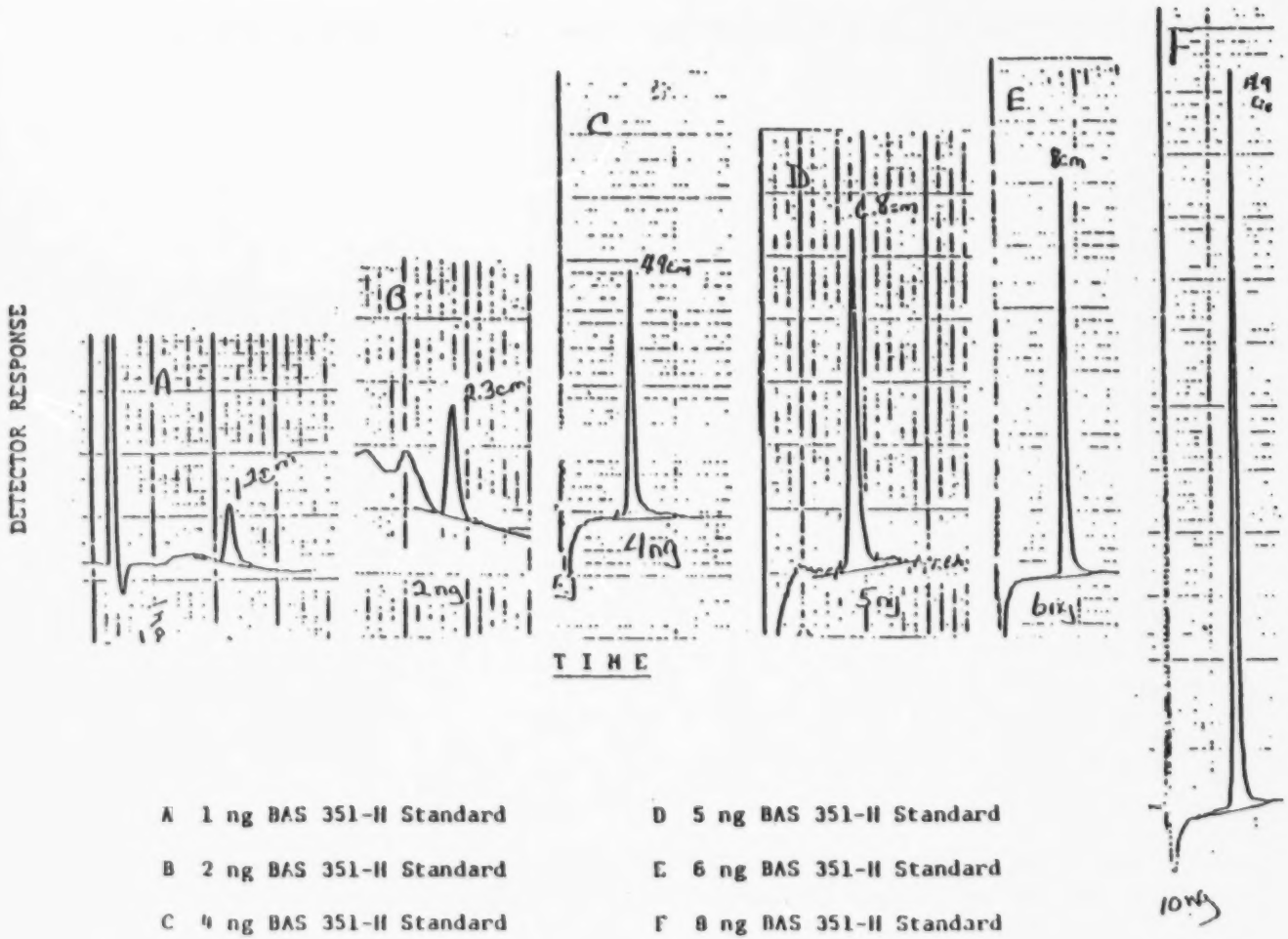


FIGURE 2

TYPICAL GLC CHROMATOGRAMS OF METHYLATED BAS 351-H STANDARDS USING FLAME PHOTOMETRIC DETECTION



A 1 ng BAS 351-H Standard

B 2 ng BAS 351-H Standard

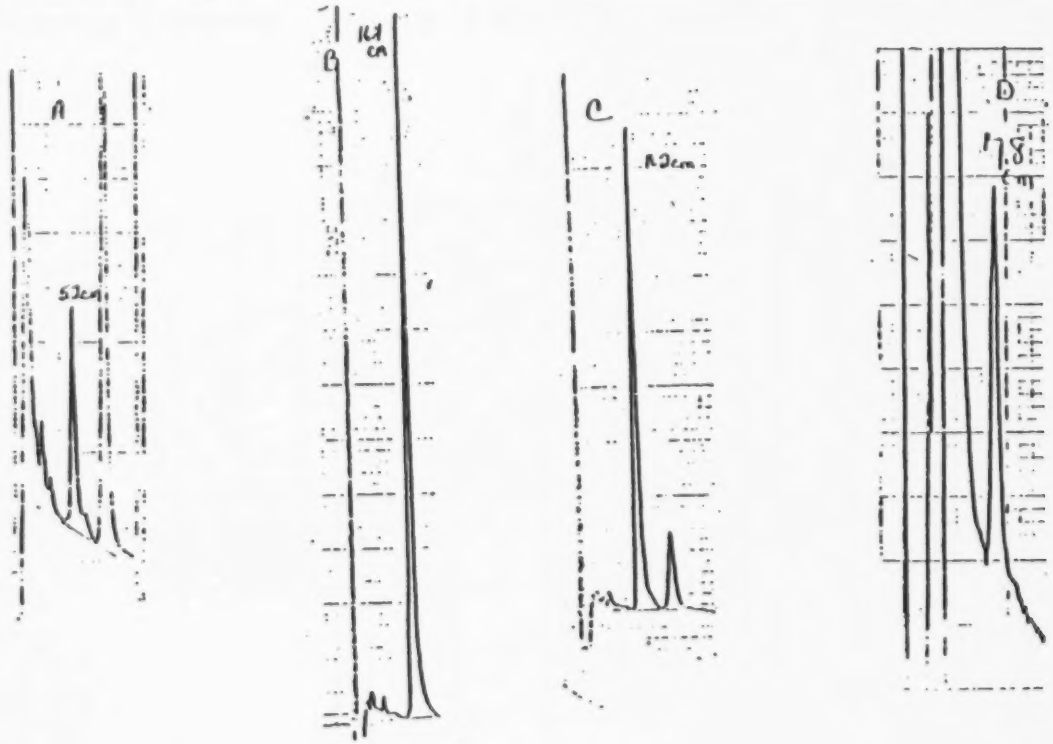
C 4 ng BAS 351-H Standard

D 5 ng BAS 351-H Standard

E 6 ng BAS 351-H Standard

F 8 ng BAS 351-H Standard

FIGURE 3 TYPICAL GLC CHROMATOGRAMS OF CONTROL, FORTIFIED CONTROL AND TREATED WATER ANALYZED FOR BAS 351-H RESIDUES USING FLAME PHOTOMETRIC DETECTION



A Control water, 500 mg injected: 4.5 ng BAS 351-H (0.01 ppm)

B Control water + 0.1 ppm BAS 351-H, 100 mg injected: 10.5 ng (105% recovery)

C Treated water, 3.0 lb/A. 125 mg injected: 8 ng BAS 351-H (0.06 ppm)

D Control water, +0.01 ppm BAS 351-H, 600 ng injected: 5.7 ng BAS 351-H (94% recovery)

Chromatograms from Experiment No. 8-A-20-73 Report No. P-9

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Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BOLSTAR IN WASTEWATER

METHOD 108

1. Scope and Application

1.1 This method covers the determination of Bolstar.

Parameter	Storet No.	CAS No.
Bolstar	--	35400-43-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater samples containing 0.005 - 1 mg/L Bolstar.

2. Summary of Method

2.1 A measured aliquot (250 mL) of wastewater is extracted with chloroform, oxidized to Bolstar sulfone using potassium permanganate, and determined by GC using a phosphorus (thermionic) detector.

3. Interferences

3.1 All glassware used for analyzing wastewater samples must be pre-rinsed with chloroform and not previously used for any analysis other than wastewater. DO NOT USE PLASTIC WASH BOTTLES FOR ANY SOLVENT.

4. Safety

4.1 Follow the EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not available.

5.2 Glassware and Other Equipment

5.2.1 Microsyringe, 10 uL

5.2.2 Rotoevaporator, Buchi or equivalent

5.3 Water bath, 50-55°C

5.4 Gas chromatograph, Varian 1400 or equivalent, equipped with a phosphorus detector (rubidium sulfate salt pellet), an 18-in x 1/8-in O.D. glass column packed with 10% DC-200 (12,500 centistokes) on Gas Chrom Q 80/100 mesh, and glass injector and detector inserts. (If the column ends are long enough to be used as inserts, the glass inserts are not necessary.)
NOTE: Use only Teflon-backed septa, Supelco No. 2-0459.

6. Reagents

6.1 Acetone, nanograde

6.2 Chloroform, nanograde

6.3 Hydrochloric acid, 1N -- Carefully add 21 mL of concentrated hydrochloric acid into a 250-mL glass-stoppered graduated cylinder containing approximately 200 mL of distilled water. Dilute to 250 mL with distilled water and mix thoroughly.

6.4 Magnesium sulfate, 20% -- Weigh 41 g of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) into a 100-mL glass-stoppered graduated cylinder. Add distilled water to the 100-mL mark, stopper, and mix.

6.5 Mineral oil solution, 2.5% -- Dilute 25 mL of mineral oil, Fisher #0-120 or equivalent, to 1000 mL with nanograde chloroform.

6.6 Bolstar sulfone (NTN 9306) standard solution, 0.10% -- Weigh 0.095-0.115 g of Bolstar (NTN 9306) sulfone (95% purity) into a 100-mL glass-stoppered graduated cylinder. Dilute to volume with acetone, stopper, and mix thoroughly. This solution may be used for one week only.

6.7 Potassium permanganate, 0.1M -- Weigh 1.6 g of potassium permanganate, ACS into a 100-mL glass-stoppered graduated cylinder. Dilute to 100 mL with distilled water and mix thoroughly.

6.8 Sodium hydroxide, 1N

6.9 Sodium hydroxide, 0.1N

6.10 Sodium sulfate, anhydrous, ACS

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Pipet a 1-mL aliquot of the 0.10% Bolstar (NTN 9306) sulfone solution into a 100-mL volumetric flask, dilute to volume with acetone, and mix thoroughly. Label this solution "10 ng". Prepare fresh daily.

7.2.2 Pipet 5-, 10-, and 20-mL aliquots of the 10-ng standard from Step 7.2.1 into separate 100-mL volumetric flasks. Dilute to volume with acetone and mix. Label these solutions 500 pg, 1000 pg, and 2000 pg, respectively. Prepare fresh daily.

8. Quality Control

8.1 Follow the EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample by shaking, then proceed immediately to Step 10.2.

10.2 Measure 250 mL of the well-mixed sample into a 500-mL separatory funnel.

10.3 If the pH of the sample is below 6.5 or above 7.0, adjust it to 6.5-7.0 with 1N sodium hydroxide or 1N hydrochloric acid using pH indicator paper or a pH meter.

10.4 Extract three times by vigorously shaking for 1 minute each time with fresh 50-mL portions of nanograde chloroform. Collect the three chloroform extracts in another 250-mL separatory funnel. If an emulsion forms, centrifuge the emulsified layer and add the clear chloroform layer, obtained by centrifuging, to the second separatory funnel. Return the water layer to the first separatory funnel.

10.5 Add 50 mL of distilled water to the combined chloroform extracts in the second separatory funnel and shake for one minute.

10.6 Filter the chloroform layer into a 300-mL 24/40 boiling flask through a funnel containing 4 to 5 g of anhydrous sodium sulfate, retained by a small glass-wool plug (pre-rinsed with 10 mL of chloroform). Rinse the sodium sulfate three times with 10 mL of chloroform, and add 10 mL of 2.5% mineral oil solution into the flask.

10.7 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.8 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.9 Add 5 mL of nanograde acetone into the sample flask. Swirl the flask to wash the inside wall with the acetone but do not allow the acetone to get on the joint or splash out.

10.10 Add 5 mL of distilled water and swirl to mix, then add 10 mL of 0.1N sodium hydroxide to the flask. Stopper, swirl to mix, and allow the flask to sit at room temperature for 15 minutes.

10.11 Add 10 mL of the magnesium sulfate solution to the flask and swirl to mix.

10.12 Add 25 mL of 0.1M potassium permanganate solution and swirl. Allow the sample to stand for 30 minutes, making sure there is an excess of potassium permanganate (as indicated by the purple color) the entire time. (Add the permanganate in 5-mL increments if necessary to maintain an excess; increase the standing time 5 minutes for each addition.)

10.13 Quantitatively transfer the sample solution from Step 10.12 into a 500-mL separatory funnel, using five 40-mL portions of distilled water.

10.14 Add 50 mL of chloroform into the separatory funnel, stopper and shake vigorously for 1 minute.

10.15 Allow the layers to separate. Drain the chloroform layer into a clean 250-mL separatory funnel.

10.16 Repeat Steps 10.14 and 10.15 twice for a total of 3 extractions.

10.17 Add 25 mL of distilled water to the chloroform extracts, stopper, and shake for 30 seconds.

10.18 Filter the chloroform layer into a 300-mL boiling flask through another funnel containing 4 to 5 g of anhydrous sodium sulfate retained by a small glass-wool plug (pre-rinsed with 10 mL of chloroform).

10.19 Rinse the sodium sulfate with three 10-mL portions of chloroform and combine with the chloroform extract.

10.20 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.21 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.22 Pipet 5 mL of acetone into the flask from Step 10.21, stopper immediately, and rotate the flask so that the acetone washes down the inside of the flask. DO NOT allow the acetone to get on the neck or stopper.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Continue the analysis according to TM A-54.12 (Mobay reference procedure, not enclosed herein).

13. Calculations

Not Available.

14. Method Performance

Not Available.

15. References

15.1 "Determination of Bolstar in Wastewater by GLC," Analytical Method TM B-34.50, Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO.

Table 1

Chromatographic Conditions

Attenuation	X32
Cell voltage	Flame
Detector temperature	230-240°C
Electrometer range	10^{-12} A
Gas flows:	
Carrier gas, Helium	30 mL/min
Air	230 mL/min
Hydrogen	40 mL/min
Injector temperature	230-240°C
Oven temperature	230°C

Column: 10% DC-200 (12,500 Centistokes) on Gas Chrom Q 80/100 mesh

Note: Conditions above are for Varian 1400 GC.

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Water and Waste Management

TEST METHOD

DETERMINATION OF BROMACIL, HEXAZINONE, OXAMYL, TERBACIL AND METHOMYL IN WASTEWATER

METHOD 109

1. Scope and Application

1.1 This method covers the determination of bromacil, hexazinone, oxamyl, terbacil, and methomyl. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Bromacil	82198	314-40-9
Hexazinone	--	51235-04-2
Oxamyl	--	23135-22-0
Terbacil	--	5902-51-2
Methomyl	39051	16752-77-5
Diuron	39650	330-54-1
Linuron	--	330-55-2

1.2 This is a liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in wastewater.

1.3 The method detection limit (MDL) for each of the pesticides is in the ug/L (ppb) range.

2. Summary of Method

2.1 Samples containing solids or a large number of interferences in the chromatogram are extracted with methylene chloride. Acetonitrile (1 mL) is added to the methylene chloride extract, which is then evaporated down to 1 mL in a 40°C water bath using a flash evaporator. The residual solution is transferred and diluted in a volumetric flask with distilled water. This solution is either injected on HPLC with UV detector or is taken through a "Sep-Pak" cleanup technique prior to HPLC analysis.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 All glassware must be cleaned thoroughly with a solvent or chromic acid to remove any trace of contamination of pesticides from the glass.

3.2 At the beginning of each day, wash the HPLC column system for ten minutes with 100% acetonitrile.

4. Safety

4.1 Methomyl is highly toxic when taken orally or inhaled as dust or mist. Wash hands before smoking or eating.

4.2 Oxamyl is highly toxic. Avoid personal contact in any manner. All pesticides should be handled with care and avoid skin contact.

4.3 Acetonitrile has a TLV of 40 ppm.

4.4 Waste streams should be handled with care, avoiding any skin contact.

4.5 See the EPA Safety procedure as found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Flash-Evaporator, Buckler Instruments, Fort Lee, NJ, which includes the condenser glassware, flasks, roto-motor, and thermostated water bath.

5.2.2 Disposable Transfer Pipets (Pasteur type), Curtin Matheson Scientific, Inc., (5 3/4" long).

5.3 Sep-Pak C-18 Cartridges, Waters Associates, Inc., Milford, MA

5.4 Sample injection valve equipped with a 3.0-mL sample loop, Rheodyne, Berkeley, CA

5.5 Du Pont Model 850 Liquid Chromatograph unit with linear gradient elution system, heated column compartment, and 254-nm UV detector, Du Pont Instrument Products Division, Wilmington, DE 19898

5.5.1 Column -- 4.6-mm ID x 25-cm prepacked "Zorbax" ODS reverse

phase column (no substitute), part number 850952702, Du Pont Instrument Products Division, Wilmington, DE 19898.

5.5.2 Printer/Plotter -- Integrator 3380A, Hewlett Packard, Avondale, PA.

6. Reagents

6.1 Acetonitrile, Burdick & Jackson, chromatographic grade (distilled in glass)

6.2 Methylene chloride, Burdick & Jackson, chromatographic grade (distilled in glass)

6.3 Bromacil, Hexazinone, Oxamyl, Terbacil, Methomyl (Dupont Biochemicals Dept.)

7. Calibration

7.1 Establish HPLC operation parameters similar to those indicated in Table 1.

7.2 Calibration Procedures

7.2.1 Analytical Stock Standard Solution

7.2.1.1 Weigh 0.0100 g ($\pm$ 0.001 g) of the analytical standards oxamyl, methomyl, bromacil, hexazinone, terbacil, diuron, linuron into separate 100-mL volumetric flasks (See Step 3.1). Note the exact weight of the standard.

7.2.1.2 Dilute to volume with distilled water. (For diuron, linuron, bromacil, and terbacil, add 5 mL acetonitrile and dilute with water after dissolution.)

7.2.1.3 Place each volumetric in an ultrasonic bath for five minutes to complete dissolution.

7.2.1.4 Store at 4°C. These solutions contain approximately 100 ug/mL of standard (solutions are stable for 4 weeks).

7.2.2 Analytical Working Standard Solutions:

7.2.2.1 Prepare 100-mL volumes of working standard solutions at 50, 250, 500, 1000, 5000, and 10,000 ug/L by diluting aliquots of the stock standard solution.

7.2.2.2 Additional working standards may be required to bracket the concentration of a pesticide in a given sample.

7.2.2.3 Store at 4°C (stable 2 weeks).

7.2.3 At 0.01 AUFS detector sensitivity, run the 50-, 250-, and 500-ug/L standards in duplicate.

7.2.4 At 0.05 AUFS recorder sensitivity run the 1000-, 5000-, and 10,000-ug/L standards in duplicate.

7.2.5 Draw calibration curves showing peak area versus concentration of standard (ug/L).

7.2.6 Additional standards may need to be run at a given detector sensitivity in order to bracket the concentration of pesticide seen in a sample.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Clean a pH probe with acetone and with water.

9.2 Using concentrated sulfuric acid or 50% sodium hydroxide, adjust the pH of the contents of each bottle to pH 6-8 within four hours after sampling. Record the volume of acid or caustic used to neutralize the sample. Final calculations should be corrected for any significant dilution of sample during neutralization.

9.3 Refrigerate the neutralized samples. Oxamyl, methomyl, and hexazinone will hydrolyze at high pH. Samples which are not neutralized will show a gradual reduction in the concentration of these pesticides. The stability of various pesticides in neutral aqueous standards at room temperature exposed to light (see Reference 1) was as follows:

	ug/L Added	ug/L Measured	Exposure Time, Days	% Recovery
Bromacil	1.0	1.00	7	100
Hexazinone	1.0	0.83	7	83
Oxamyl	10	8.2	14	82
Methomyl	100	95	7	95
Terbacil	1.0	0.99	7	99
Diuron	10	9.3	7	93
Linuron	10	9.6	7	96

9.4 See the EPA sample collection procedure as found in Part D of this document.

10. Sample Extraction

10.1 Mark the level of the water sample on the side of the glass bottle (Step 10.14).

10.2 Pour the contents of the bottle into a separatory funnel.

10.3 Wash the bottle with 60 mL of methylene chloride and transfer it to the separatory funnel.

10.4 Shake the separatory funnel for two minutes. Avoid forming an emulsion by shaking gently.

10.5 Transfer the methylene chloride layer into a clean 250-mL flash-evaporator flask, leaving a small portion (approximately 1 mL) of solvent in the funnel.

10.6 Repeat Steps 10.3, 10.4 and 10.5 two more times.

10.7 Discard the water layer.

10.8 Add 1 mL of acetonitrile to the flask containing the methylene chloride extract.

10.9 Place the flask into the 40°C water bath and connect it onto the flash-evaporator. Evaporate until only 1 mL of acetonitrile remains.

10.10 If the sample requires no Sep-Pak cleanup, transfer the residual solution into a volumetric flask (Step 10.14) using a clean glass funnel and three 5-mL washes of the evaporator flask with distilled water.

10.11 Dilute to volume with distilled water. This solution is ready for HPLC analysis. Record the volume to calculate the concentration factor.

10.12 If the sample requires Sep-Pak cleanup, add 200 mL of distilled water to the evaporator flask and proceed with the Sep-Pak procedure.

10.13 Using a graduated cylinder, measure the volume of water required to refill the original sample bottle to the mark and record the volume.

10.14 Sample Information

Sample Identification	Technique Used	Detector Sensitivity (AUFS)	Attenuation
Outfall 101	500 mL extracted with methylene chloride, final volume of 25 mL of water for injection on HPLC	0.01 for 15 min. 0.05 to end	8
Lift Station Effluent (LSE)	500 mL extracted with methylene chloride, final volume of 25 mL of water for injection of HPLC	0.05	8
Central Scrubbed Incinerator Feed (CSIF)	100 mL extracted with methylene chloride, Sep-Pak cleaned, final volume of 100 mL of water for injection on HPLC	0.05	8
Central Scrubbed Incinerator Purge (CSIP)	Direct injection of this sample on HPLC	0.01	1
Chemical Oxidation Feed	Direct injection of this sample on HPLC	0.05	8
Chemical Oxidation Discharge	Direct injection of this sample on HPLC	0.01	8

11. Cleanup and Separation

11.1 Prepare the Sep-Pak cartridge train as follows:

11.1.1 Connect two Sep-Pak cartridges using a short piece of polyethylene tubing. (Do not use Tygon tubing.) The short end of one Sep-Pak should be connected to the long end of the other Sep-Pak.

11.1.2 Connect the long end of the Sep-Pak train to a disposable pipet. The Sep-Pak tubing will fit inside the inner diameter of the disposable pipet.

11.1.3 Connect the short end of the Sep-Pak train to a vacuum flask using flexible tubing.

11.1.4 To wash the Sep-Pak train, place the disposable pipet into 10 mL of acetonitrile allowing the vacuum to pull the solvent through the Sep-Pak.

11.1.5 Repeat Step 11.1.4 with 10-15 mL of distilled water. The Sep-Pak train is now ready for use on a sample cleanup.

11.2 Place the Sep-Pak train into the evaporator flask (See Step 10.12), allowing the vacuum to pull 200 mL of aqueous solution through the Sep-Paks.

11.3 Turn the Sep-Pak train upside down to allow the vacuum to remove as much water as possible from the train.

11.4 Disconnect the Sep-Pak train from the vacuum flask tubing.

11.5 Put the disposable pipet end of the train into a volumetric flask.

11.6 Connect a 10-mL syringe with a Luer end fitting to the Sep-Pak train.

11.7 Remove the plunger from the syringe. Pour 10 mL of acetonitrile into the syringe barrel and replace the plunger. Push the acetonitrile through the Sep-Pak train into the volumetric flask.

11.8 Repeat step 11.7 with air in the syringe to displace any residual acetonitrile into the flask.

11.9 Place the volumetric flask in a water bath at 65°C-70°C. Use a nitrogen purge to aid in evaporation of the acetonitrile down to 1 mL. Dilute to volume with distilled water. The sample is now ready for HPLC. Record the volume to calculate the concentration factor.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph

12.2 Run a gradient baseline at the beginning of each day

12.3 HPLC Injection Technique

12.3.1 With the sample valve in the LOAD position, inject 3-4 mL of sample through the sample loop with a 5-mL syringe.

12.3.2 Inject the sample by turning the sample valve to the INJECT position. Depress the RUN button on the LC.

12.3.3 Depress the START/STOP button on the printer/plotter.

12.3.4 Settings on printer/plotter

a.	Report	- Method
b.	Start Delay	- OFF
c.	Stop Timer	- 60 min
d.	Area Reject	- 100
e.	Chart Speed	- 0.5 cm/min
f.	Chart	- AUTO
g.	Slope Sensitivity	- 0.3 mV/min

h. Attenuation - See Step 10.14

12.3.5 Each sample or standard should be run in duplicate.

13. Calculations

13.1 From the calibration curves, determine the concentration (ug/L) of pesticide in the injected sample.

13.2 If the sample was concentrated by extraction or Sep-Pak cleanup, determine the concentration (ug/L) of pesticide in the original sample as follows:

$$\text{Concentration (ug/L) in original sample} = \frac{\text{ug/L in injected sample}}{\text{concentration factor}}$$

$$\text{Concentration factor} = \frac{\text{mL of original sample}}{\text{mL of final volume after extraction and/or cleanup}}$$

13.3 The concentrated sample may have to be diluted to bring the peak area within the linear range of the calibration curve for some pesticides. In this case, calculations are as follows:

Concentration (ug/L) in original sample =

$$\frac{\text{ug/L in injected sample}}{\text{concentration factor}} \times \text{dilution factor}$$

$$\text{Dilution factor} = \frac{\text{mL of final diluted volume}}{\text{mL of concentrate taken}}$$

14. Method Performance

14.1 A detector sensitivity of 0.01 to 0.05 AUFS was used depending on which sample was being analyzed. The sensitivity for all of the subject pesticides is in the ug/L range.

14.2 Precision and accuracy data have not been determined.

14.3 Methylene chloride extraction is used only if the sample contained solids or if it requires "Sep-Pak" cleanup. The extraction efficiencies for various pesticides are as follows (see Reference 1):

	ug/L Added	ug/L Measured	% Recovery
Bromacil	1	0.91	91
Hexazinone	1	1.02	102
Oxamyl	10	9.8	98
Methomyl	100	78	78
Terbacil	1	0.97	97
Diuron	10	9.0	90
Linuron	10	9.1	91

14.4 The % recoveries of the subject pesticides using the "Sep-Pak" cleanup technique are as follows:

	% Recovery for a Given Amount of Pesticide				
	1.25 ug	25 ug	500 ug	2500 ug	5000 ug
Bromacil	103	91	-	-	-
Hexazinone	98	102	-	-	-
Oxamyl	101	98	-	87	-
Methomyl	81	76	-	-	-
Terbacil	126	101	-	106	92
Diuron	97	98	101	-	-
Linuron	92	98	98	-	-

15. References

15.1 Midwest Research Institute, Pesticide Analysis Methods. A contract study conducted by MRI for the U.S. Environmental Protection Agency.

15.2 "Determination of Houston Plant Pesticides, High Pressure Liquid Chromatography (HPLC) Method," Method No. W28.303(R), April 29, 1980, E.I. duPont de Nemours & Co., Inc. Houston, TX.

Table 1

Chromatographic Conditions

Column temperature	35°C
Detector	254-nm fixed wavelength
Detector sensitivity	0.01 to 0.05 Absorbance Units Full Scale (AUFS)
Flow rate	2.0 mL/min
Maximum Pressure	100 Bar
Sample Size	2.0 mL, nominally
Gradient	Program
Water	Solvent A
Acetonitrile	Solvent B
a. Step 1 0% B to 40% B	40 min
b. Step 2 40% B to 100% B	8 min
c. Step 3 100% B to 100% B	3 min
d. Step 4 100% B to 0% B	5 min
e. Step 5 0% B to 0% B	5 min

Column: 4.6-mm ID x 25-cm prepacked "Zorbax" ODS reverse phase column.

Table 2

Retention Times

Compound	Retention Time (Minutes)
bromacil	31.6
hexazinone	33.2
oxamyl	15.5
methomyl	16.6
terbacil	32.7
diuron	41.8
linuron	46.7

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Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BUSAN 4C, BUSAN 85, AND KN-METHYL IN WASTEWATER

METHOD 110

1. Scope and Application

1.1 This method covers the determination of certain dithiocarbamates. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Busan 40	--	51026-28-9
Busan 85	--	128-03-0
KN-Methyl	--	--

1.2 This method determines the amount of total dithiocarbamates present. It cannot distinguish one compound from another.

1.3 This is a spectrophotometric method applicable to the determination of the compounds listed above in water.

2. Summary of Method

2.1 The absorbance of the sample is measured at 284 nm. The absorbance value indicates the maximum level of dithiocarbamates present, and a sufficiently low value shows that little or no dithiocarbamates are present.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

Not Available.

6. Reagents

Not Available.

7. Calibration

7.1 Measure the absorbance of the sample solution at 284 nm in a 1-cm cell.

7.2 For Busan 40, Busan 85, and KN-methyl, the following absorbance values will indicate 1 ppm of analyte present in the solution:

	$A_{1\ ppm,\ 284\ nm,\ 1\ cm}$
Busan 40	0.062
Busan 85	0.066
KN-methyl	0.071

7.3 Using these values, an absorbance value of 0.010 under the indicated conditions will represent the following levels of analyte:

	ppm
Busan 40	0.16
Busan 85	0.15
KN-methyl	0.14

7.4 As previously indicated, these are maximum values and the method of Lowen and Pease (1964) must be used if verification is desired.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

Not Available.

11. Cleanup and Separation

Not Available.

12. Sample Analysis

12.1 Dithiocarbamates may be measured in water by measuring the absorbance at 284 nm. The absorbance value will indicate the maximum level of dithiocarbamate present, and a sufficiently low value will show that little or no dithiocarbamate is present. If significant levels are indicated, the sample must be analyzed by the method described by Lowen and Pease in "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," Volume III, Fungicides, Nematocides and Soil Fumigants, Rodenticides, and Food and Feed Additives, Chapter 7, pages 69-77, edited by G. Sweig, Academic Press, New York, 1964.

13. Calculations

Not Available.

14. Method Performance

Not Available.

15. References

15.1 "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," Vol III, Fungicides, Nematocides and Soil Fumigants, Rodenticides, and Food and Feed Additives, Chapter 7, pages 69-77, 1964.

15.2 "Analysis of Effluent for Dithiocarbamates," Standard Test Method, Buckman Laboratories, Inc., Memphis, TN.

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TEST METHOD

DETERMINATION OF CARBOFURAN
N WASTEWATER

METHOD 111

1. Scope and Application

1.1 This method covers the determination of carbofuran.

Parameter	Storet No.	CAS No.
Carbofuran	81405	1563-66-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 A measured aliquot of sample (100 mL) is extracted with methylene chloride. The extract is concentrated to 10 mL on a hot water bath with dry air or nitrogen. Analysis is by gas chromatography with flame ionization detector (FID).

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnels, 250 mL, 60° funnels, 70-mm narrow stem

5.2.2 Erlenmeyer flasks, 250 mL, 500 mL

5.2.3 Volumetric flasks, 100 mL

5.2.4 Graduated Centrifuge tubes, 13 mL

5.3 Whatman Filter Paper, No. 1

5.4 Nuchar - Attaclay - Kensington Scientific Corp.

5.5 Hot Water Bath

5.6 Gas Chromatograph with FID detector

5.6.1 Column: 3-ft x 1/8-in O.D. SS packed with 5% OV-17 on Chromosorb W-HP

5.6.2 HP 3380 A Integrator or similar

6. Reagents

6.1 Na_2SO_4, reagent grade anhydrous

6.2 Distilled in glass solvents from Burdick & Jackson or similar

6.2.1 Methylene Chloride

6.2.2 Chloroform

6.2.3 Ethyl Acetate

6.2.4 Hexane

6.3 Filtered dry air supply

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 0.1000 g carbofuran standard into a 100-mL volumetric flask. Dilute to volume with chloroform (1.0 ug/uL).

7.2.2 Pipet 10 mL of the 1-ug/uL standard into a 100-mL volumetric flask and dilute to volume with chloroform (0.1 ug/uL).

7.2.3 Make several 1-uL injections of the 0.1-ug/uL standard into the gas chromatograph with FID detection system. Determine the area of the carbofuran peak. This area represents 0.1 ug.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Prewash Na_2SO_4 with methylene chloride to remove contaminants.

10.2 Place No. 1 Whatman paper in 60° funnel and fill 3/4 full with Na_2SO_4. Wash Na_2SO_4 with three 25-mL portions of methylene chloride. Discard the washings collected in the flask.

10.3 Extract 100 mL of water sample with three 25-mL portions of methylene chloride, passing each portion through the prewashed Na_2SO_4 into the Erlenmeyer flask (500 mL).

10.4 Wash down the Na_2SO_4 with another three 25-mL portions of methylene chloride for a total of 125 mL in flask.

10.5 Concentrate the extract to 10 mL on a hot water bath with a gentle stream of dry air or nitrogen.

10.6 Transfer the concentrate from the flask to a 13-mL graduated centrifuge tube. Rinse the flask with 3 mL chloroform and transfer to the centrifuge tube. Concentrate to less than 1.0 mL using a gentle stream of dry air. Dilute the sample to a final volume of exactly 1.0 mL with chloroform.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Inject a suitable aliquot (about 1-2 uL) of extracted water sample.

13. Calculations

13.1 $\frac{\text{Area (unk)}}{\text{Area (std)}} \times 0.1 \text{ ug} = \text{amount of Carbofuran in aliquot (ug)}$

13.2 $$\frac{\text{ug's of Carbofuran} \times \frac{\text{Total extract vol. (uL)}}{\text{Vol. injected (uL)}}}{\text{Sample wt. (100 g if 100 mL water extracted)}} = \text{ug/g (ppm) Carbofuran}$$

14. Method Performance

Not Available.

15. References

15.1 "Gas Chromatographic Analysis of Carbofuran in Water at PPM Level," Standard Test Method, Middleport Control Laboratory, FMC Agricultural Chemical Division, Middleport, NY.

Table 1

Chromatographic Conditions

Column Temperature	175°C
Injection Temperature	200°C
Detector Temperature	250°C
Flow Rate: Carrier	He, 25-30 mL/min
Detector	H_2, 25-30 mL/min
Air	350-400 mL/min
Detector: Range	10
Attenuation	4

H.P. 3380 A Integrator or similar

Start Delay - 1 min.
Stop Delay - 10 min.
Area Reject - 100
Chart Speed - 0.5 cm/min.
Chart - Auto
Slope Sensitivity - .03
Attn - 1

Column: 3-ft x 1/8-in O.D. S.S. packed with 5% OV-17 on Chromosorb W-HP

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TEST METHOD

DETERMINATION OF CHLOROBENZILATE IN WASTEWATER

METHOD 112

1. Scope and Application

1.1 This method covers the determination of chlorobenzilate. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Chloropropylate	—	5836-10-2
Chlorobenzilate	39460	510-15-6

1.2 This is a thin-layer chromatographic (TLC) method applicable to the determination of the compounds listed above in wastewater.

1.3 The method detection limit (MDL) is 0.5 mg/L.

2. Summary of Method

2.1 A measured volume of water sample (50 mL) is extracted with methylene chloride. The extract is analyzed by silica gel TLC. Spot development is with $AgNO_3$ and visualization is by UV light.

3. Interferences

Not Available.

4. Safety

4.1 Plate spraying and exposure to UV radiation should be carried out in a hood. Special glasses must be used when working with UV light. Eyes and skin must be protected from UV radiation.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling equipment

Not Available.

5.2 Plate: Silica Gel G, neutral (50% Merck Silica Gel G, 50% Bio Rad Bio Sil-A) on 200- x 200-mm glass plates coated at a thickness of 200 u.

5.3 Chamber: Saturated
Chamber size - 25 cm x 29 cm x 10 cm

6. Reagents

6.1 Silica Gel G

6.2 Chloroform

6.3 Chloropropylate

6.4 Chlorobenzilate

6.5 Eluent n-hexane/3-A alcohol (anhydrous) 95/5 (by volume)

7. Calibration

7.1 Normal principles of thin-layer chromatography apply for this analysis. Table 1 gives TLC conditions.

7.2 Calibration Procedure

7.2.1 Weigh 0.0100 g each of chloropropylate and chlorobenzilate of 99+% purity into a 100-mL volumetric flask. Dilute this stock standard to the mark with $CHCl_3$ and carry out a serial dilution to the third dilution. These solutions are equivalent to 4, 2, 1, and 0.5 mg/L when compared to samples extracted 5:1 with samples applied 5 x and standards applied 1 x.

7.2.2 Five applications of samples (approximately 35 uL total) drying between increments. One application of standards using open-end capillary tubes (7-8 uL). Capillary tubes from Kimax size 1.6-1.8 x 100 mm.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Extract a 50-mL sample with 10 mL of chloroform in a 125-mL separatory funnel. Isolate the chloroform layer for spotting.

11. Cleanup and Separation

Not Available.

12. Thin-Layer Chromatography

12.1 Table 1 summarizes the recommended operating conditions for TLC.

12.2 Calibrate the system as described in Section 7.

12.3 After elution, air dry plate to remove solvents. Then spray with 0.02N $AgNO_3$ and expose to 1400-watt, 366-nm maximum wavelength ultraviolet light until maximum contrast is achieved. (DANGER - PROTECT EYES AND SKIN FROM UV RADIATION.) Estimations are made by visual comparisons between samples and appropriate standards.

12.4 A fresh eluent should be prepared each day and allowed to equilibrate 1 hour before use.

12.5 Fresh standard solutions should be prepared once per month.

13. Calculations

Not Available.

14. Method Performance

14.1 A statement of "not detected" (ND) for a component indicates only that the component is less than 0.5 ug/L.

14.2 The standard deviation of an analysis is approximately 30% relative to actual concentration.

15. References

15.1 "TLC Determination of Chloropropylate and Chlorobenzilate in Wastewater," ETM-36-1, June 6, 1977, Ciba-Geigy, AL.

Table 1

Chromatographic Conditions

Eluent	n-hexane/3-A alcohol (anhydrous) 95/5 (by volume)	
Chamber:		
	Saturated Chamber size	25 cm x 29 cm x 10 cm
Distance		15 cm
Time		40 minutes

Table 2

Typical Separation Rf

Rf	Compound
0.6	Chloropropylate
0.5	Chlorobenzilate
0.0	Point of origin

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TEST METHOD

DETERMINATION OF CHLORPYRIFOS AND CHLORPYRIFOS METHYL IN WASTEWATER

METHOD 113

1. Scope and Application

1.1 This method covers the determination of chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] and chlorpyrifos methyl.

Parameter	Storet No.	CAS No.
Chlorpyrifos	81403	2921-88-2
Chlorpyrifos methyl	--	5598-13-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in wastewater.

1.3 The method detection limit (MDL) for chlorpyrifos is 0.001 ug/g (ppm).

2. Summary of Method

2.1 A measured water sample (50 mL) is extracted with methylene chloride. The methylene chloride extract is evaporated to dryness with a jet of dry air. The residue is redissolved in a known volume of acetone, and an aliquot is taken to analyze for the presence of chlorpyrifos by gas chromatography, with flame photometric detection.

3. Interferences

Not Available.

4. Safety

4.1 Acetone is flammable and should be used in well ventilated areas away from an ignition source.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Syringe, 10-uL, Hamilton brand No. 701N or equivalent.

5.2.2 Separatory funnels, 125-mL, with stopcock made of Teflon resin and glass stoppers, Corning No. 6402.

5.2.3 Round bottles, 2-oz, with Polyseal caps.

5.3 Evaporation manifolds (see Figure 1)

5.4 Strip chart recorder, Hewlett-Packard Model 7137 or equivalent

5.5 Gas chromatograph, Micro Tek brand, Model MT-160 or equivalent, equipped with a flame photometric detector, Tracor, Inc., Austin, TX 78221

5.5.1 Flame photometric detector, Melpar brand, Model FPD100AT with a 526-mu interference filter (selective, sensitive response to phosphorus). Tracor, Inc., Austin, TX.

5.5.2 Gas chromatographic column: loop-shaped borosilicate glass, 6-ft x 5/32-in I.D. x 1/4-in O.D.

5.5.3 The glass heat shields (windows) in the flame photometric detector should be checked at least once a month for clearness and changed as needed

6. Reagents

6.1 Methylene chloride, distilled in glass, Burdick & Jackson Laboratories, Inc., Muskegon, MI

6.2 Acetone, nanograde, Mallinckrodt Chemical Works, St. Louis, MO

6.3 OV-17 and QF-1 (mixed phase), 11% on 80/100 mesh Gas-Chrom Q; Cat. No. 12970, Applied Science Laboratories, Inc., P.O. Box 440, State College, PA 16801

6.4 Chlorpyrifos, O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate, analytical standard, Dow Chemical U.S.A., Midland, MI

6.5 Prepurified nitrogen (carrier gas), Air Reduction Company.

6.6 Hydrogen (burner gas), Air Reduction Company

6.7 Oxygen (burner gas), Air Reduction Company

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Calibration and spiking standards -- Transfer 0.1000 g of chlorpyrifos analytical standard to a 100-mL volumetric flask and dissolve in acetone. Dilute to the 100-mL mark to obtain a 1000-ug/mL stock solution. Prepare working standards as shown in Table 2.

7.2.2 Injection Technique

7.2.2.1 Fill the 10-uL syringe with the solution to be injected, eliminating all air bubbles with rapid up-down strokes.

7.2.2.2 Inject the sample into the chromatographic column with rapid injection rate and rapid withdrawal of the needle from the column.

7.2.2.3 Turn the 4-port valve to the vent position for approximately 25 seconds immediately after sample injection in order to vent solvent and prevent extinguishing flame, then close.

7.2.2.4 Change septum in the injection part of the chromatograph daily when in use.

7.2.2.5 The glass insert in the injection part should be replaced with a clean, dry insert at least once a week when in daily use.

7.2.3 Inject a 10-uL aliquot of the chlorpyrifos standard solutions, covering the concentration range from 0.05 to 0.5 ug/mL, into the chromatograph and record the resulting peak heights. Plot peak heights on the ordinate as percent full-scale deflection vs nanograms of chlorpyrifos on the abscissa. A typical standard curve is presented in Figure 2.

8. Quality Control

8.1 Mix a freshly-thawed control sample of water.

8.2 Pipet 50-mL aliquots of the water sample into a series of 125-mL separatory funnels.

8.3 Use a portion of the weighed samples as controls, and fortify the remaining samples by adding 0.5-mL aliquots of 0.1-, 1-, 10-, and 100-ug/mL standard solutions of chlorpyrifos to obtain

concentrations ranging from 0.001 to 1.0 ppm. Prepare each sample in duplicate.

8.4 Add 25 mL of methylene chloride to each sample contained in a 125-mL separatory funnel.

8.5 Shake each separatory funnel vigorously by hand for 1 minute, allow the separatory funnel to stand for 30 seconds with venting, and again shake vigorously by hand for 1 minute.

8.6 Allow the phases to separate and drain the lower methylene chloride layer into a 2-oz bottle.

8.7 Repeat steps 8.4, 8.5, and 8.6 three more times using 20, 15, and 10 mL of methylene chloride, and collect each extraction in the same 2-oz bottle.

8.8 Evaporate the combined extractions to dryness with a gentle jet of dry air and a water bath at about 50°C.

8.9 Dissolve the residue in 1 mL of acetone and cap the 2-oz bottle with a Polyseal cap. Shake the bottle to dissolve the residue.

8.10 Chromatograph a 10-uL aliquot of the acetone solution as described in Step 7.2.2. (Dilute and re-inject if necessary.) Measure the height of the peak obtained for chlorpyrifos in terms of percent full-scale deflection and determine the weight of chlorpyrifos injected (in nanograms) by reference to a standard curve derived on the same day (see Step 8.13).

8.11 Correct for blank, if any, by subtracting the concentration of apparent chlorpyrifos in the control sample from that of the treated sample.

8.12 Calculate percent recovery of chlorpyrifos from each of the fortified samples and average the results.

8.13 Chlorpyrifos elutes from the gas chromatography column with a retention time of about 1.4 minutes. The lower detection limit of chlorpyrifos is about 0.25 ng. The most accurate calibration technique is to bracket each sample injection with an injection of a standard that closely approximates its concentration and then average the responses of the standard injections to calculate the concentration of the sample solution.

8.14 See EPA Quality Control procedure found in Part D of this document.

9. <u>Sample Collection, Preservation, and Handling</u>

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. <u>Sample Extraction</u>

10.1 Analyze 50-g samples of water from the control and treated samples as described in Section 8, Steps 8.1 through 8.9.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for GC.

12.2 Fill the GC column with packing with the aid of a slight vacuum. Place a piece of cheesecloth between the effluent end of the column and the vacuum hose to hold the packing in the column. Tap the sides of the column gently until no further settling occurs. Remove the packing from the first 1/2 inch of each end of the column with a wire and insert a small glass-wool plug in, close each end. Condition the column at 230°C overnight with a nitrogen flow of above 200 mL/min and the 4-port valve in the vent position.

12.3 Calibrate the system as described in Section 7.

12.4 Inject the sample extract into the GC as described in Step 7.2.2.

12.5 Record the peak heights for each sample.

12.6 Typical chromatograms of chlorpyrifos standards and extracts of unfortified and fortified water are shown in Figures 3 through 5.

13. Calculations

13.1 Determine the ppm chlorpyrifos in each treated sample as follows:

$$\text{Gross ppm} = \frac{(\text{ng})(10^{-3}\ \text{ug/ng})\left(\frac{\text{Final Dilution, ml}}{0.010\ \text{mL}}\right)}{\text{Sample Weight, g}}$$

Net ppm = gross ppm in sample - gross ppm in control

$$\text{Apparent ppm} = \frac{\text{net ppm}}{\%\ \text{Recovery}} \times 100$$

14. Method Performance

14.1 Recovery of chlorpyrifos from deionized water fortified over the range of 0.001 to 1.0 mg/L averaged 92 ± 5% at 95% confidence limits for the mean. See Table 3.

15. References

15.1 Wetters, J.H. and Dishburger, H.J., "Determination of Residue of 0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate in Water by Gas Chromatography with Flame Photometric Detection," ACR 71.21, Dow Chemical U.S.A., Midland, MI.

Table 1

Chromatographic Conditions

Column temperature	205°C
Injection block temperature	220°C
Detector temperature	215°C
Carrier gas and make-up gas	prepurified N_2 at 200 mL/min
Burner gas	H_2 at 200 mL/min
Burner gas	O_2 at 30 mL/min
Recorder	0-1 mv.
Electrometer sensitivity	input attenuator 10^3, output attenuator 64.
Chart speed	0.25 in/min.

Table 2

Preparation of Working Standards

Conc. of Standard, ug/mL	Alq., mL	Diluted to Volume, mL	Conc. of Solution, ug/mL
1000	10	100	100
100	10	100	10
10	10	100	1
10	5	100	0.5
10	4	100	0.4
10	3	100	0.3
10	2	100	0.2
1	10	100	0.1
1	5	100	0.05

Table 3

Recovery of Chlorpyrifos from Water Samples
Fortified with Known Amounts of the Compound

mg/L (ppm) Added	mg/L (ppm) Found	% Recovery
0.001	0.00089	89
0.001	0.00093	93
0.001	0.00097	97
0.001	0.00110	110
0.01	0.0085	85
0.01	0.0087	87
0.01	0.0069	69
0.01	0.0089	89
0.1	0.097	97
0.1	0.097	97
0.1	0.092	92
0.1	0.089	89
1.0	1.01	101
1.0	0.94	94
		92 ± 5*

* 95% confidence limits for the mean.

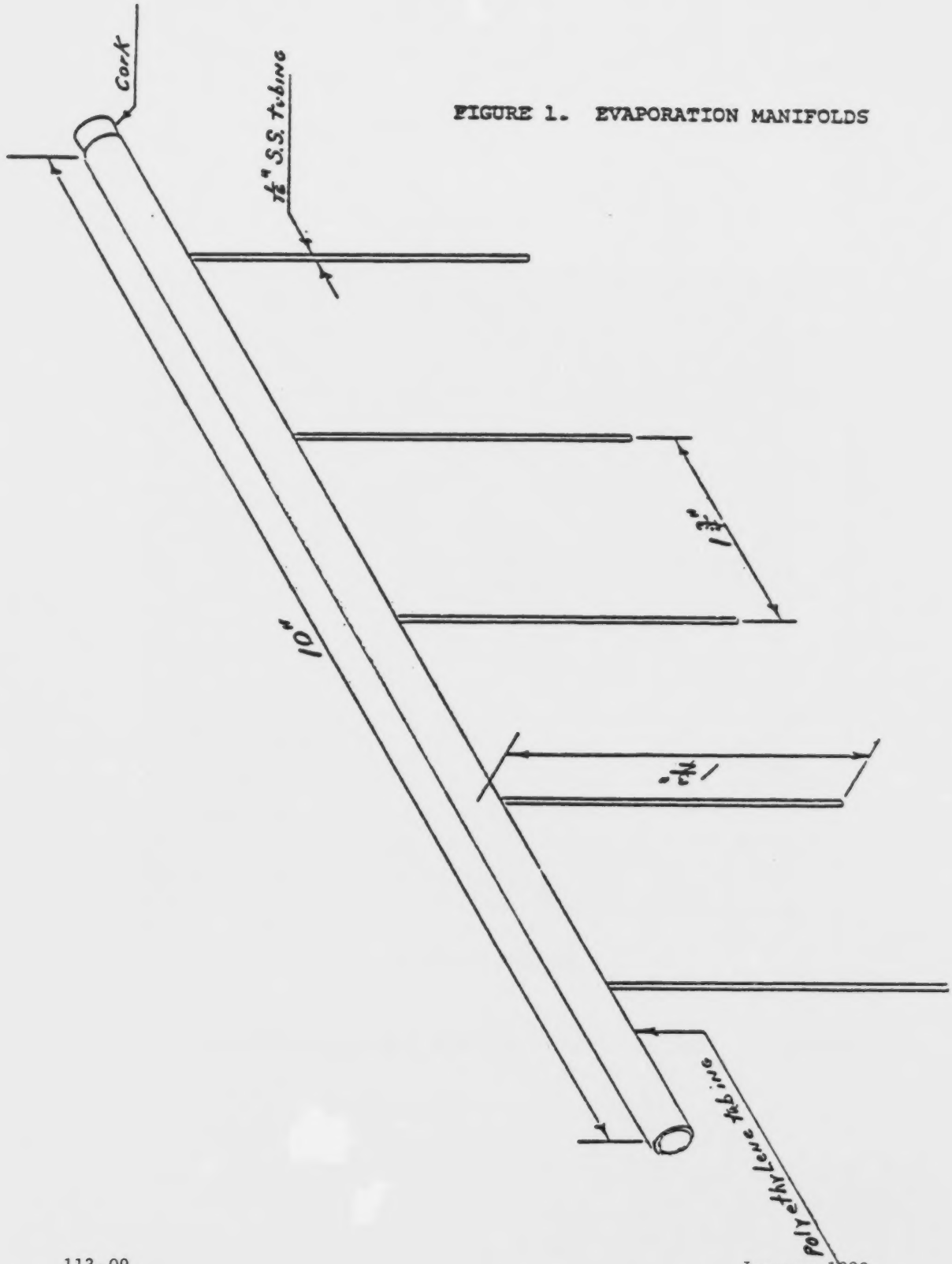


FIGURE 1. EVAPORATION MANIFOLDS

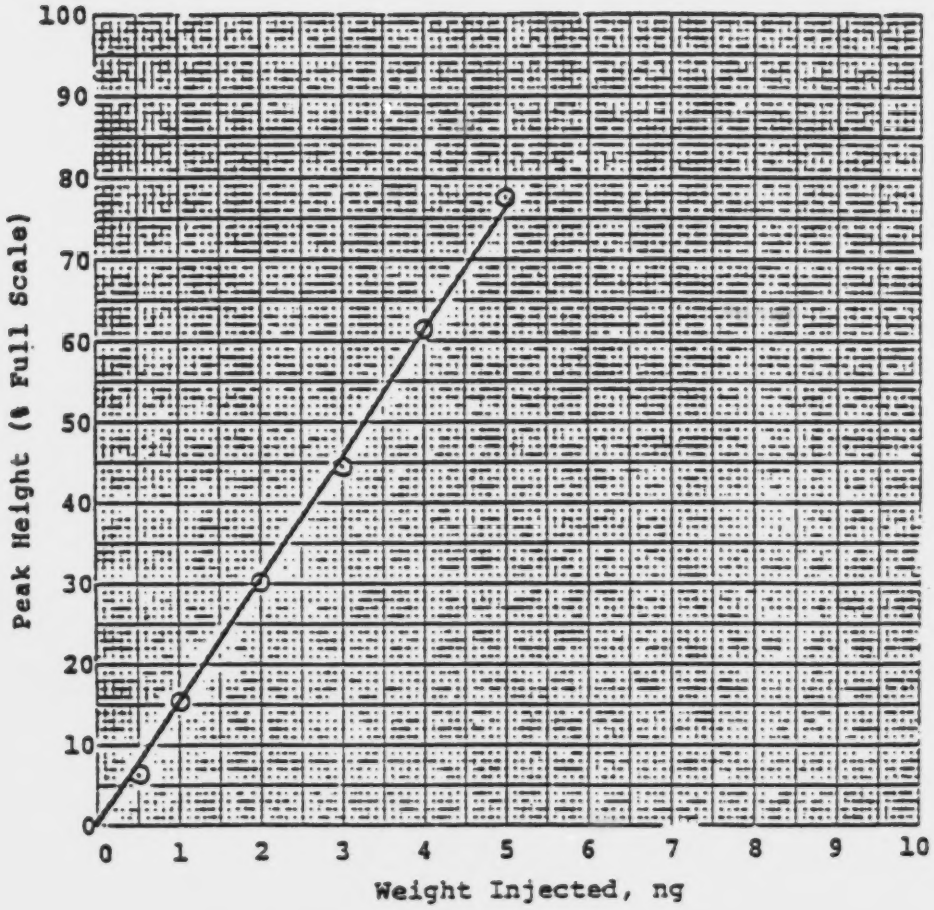
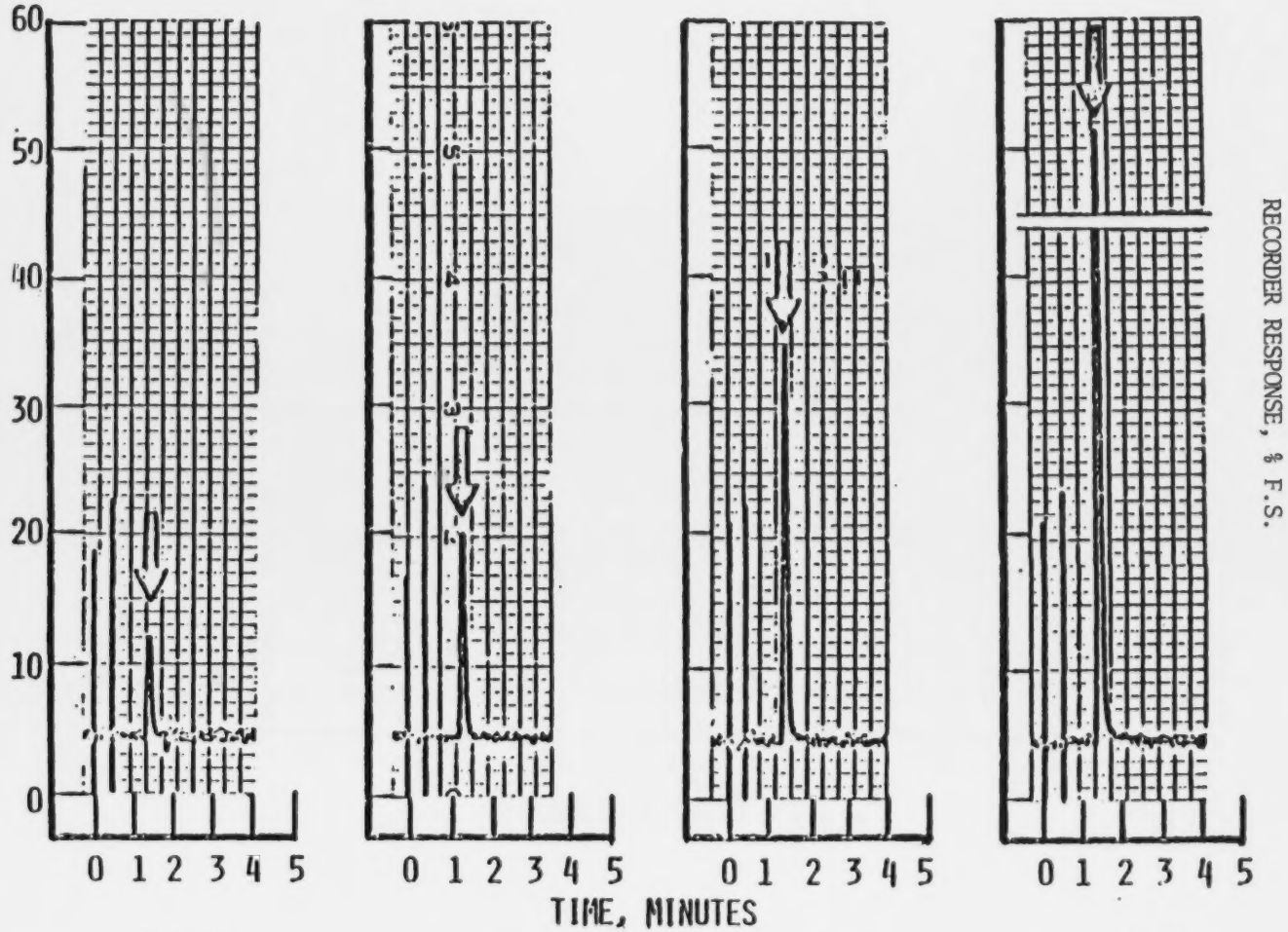


FIGURE 2. TYPICAL STANDARD CURVE FOR CHLORPYRIFOS.



0.5 ng Equivalent to 0.001 ppm	1 ng Equivalent to 0.002 ppm	2 ng Equivalent to 0.004 ppm	4 ng Equivalent to 0.008 ppm

FIGURE 3. TYPICAL CHROMATOGRAMS OF CHLORPYRIFOS STANDARD SOLUTIONS.

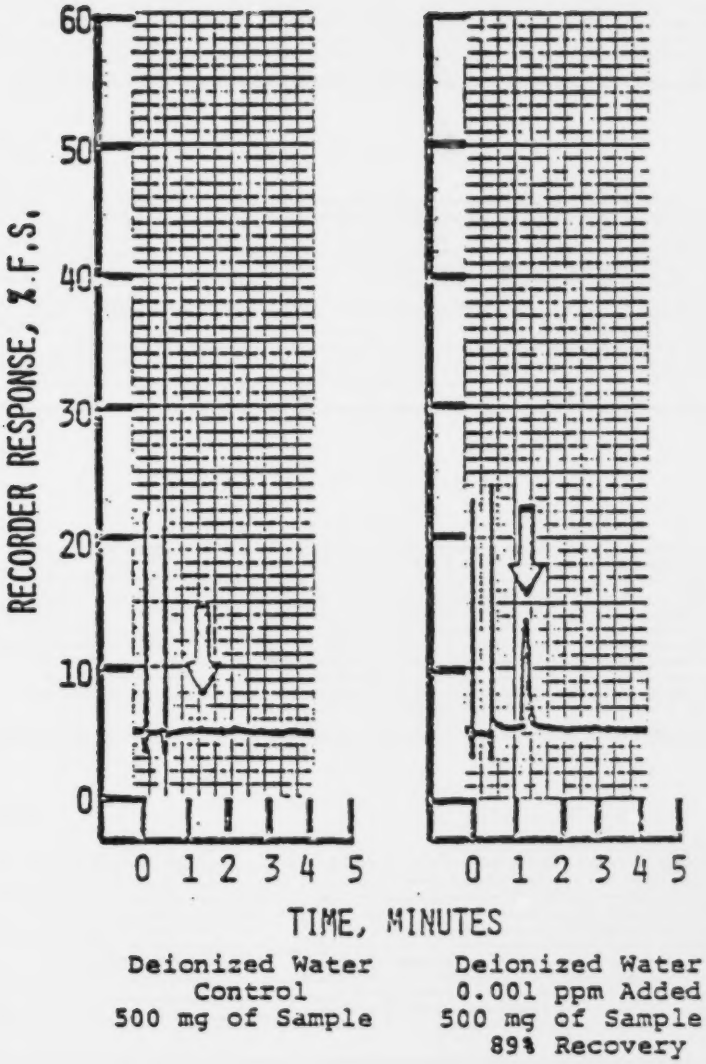


FIGURE 4. TYPICAL CHROMATOGRAMS OF WATER FORTIFIED WITH CHLORPYRIFOS.

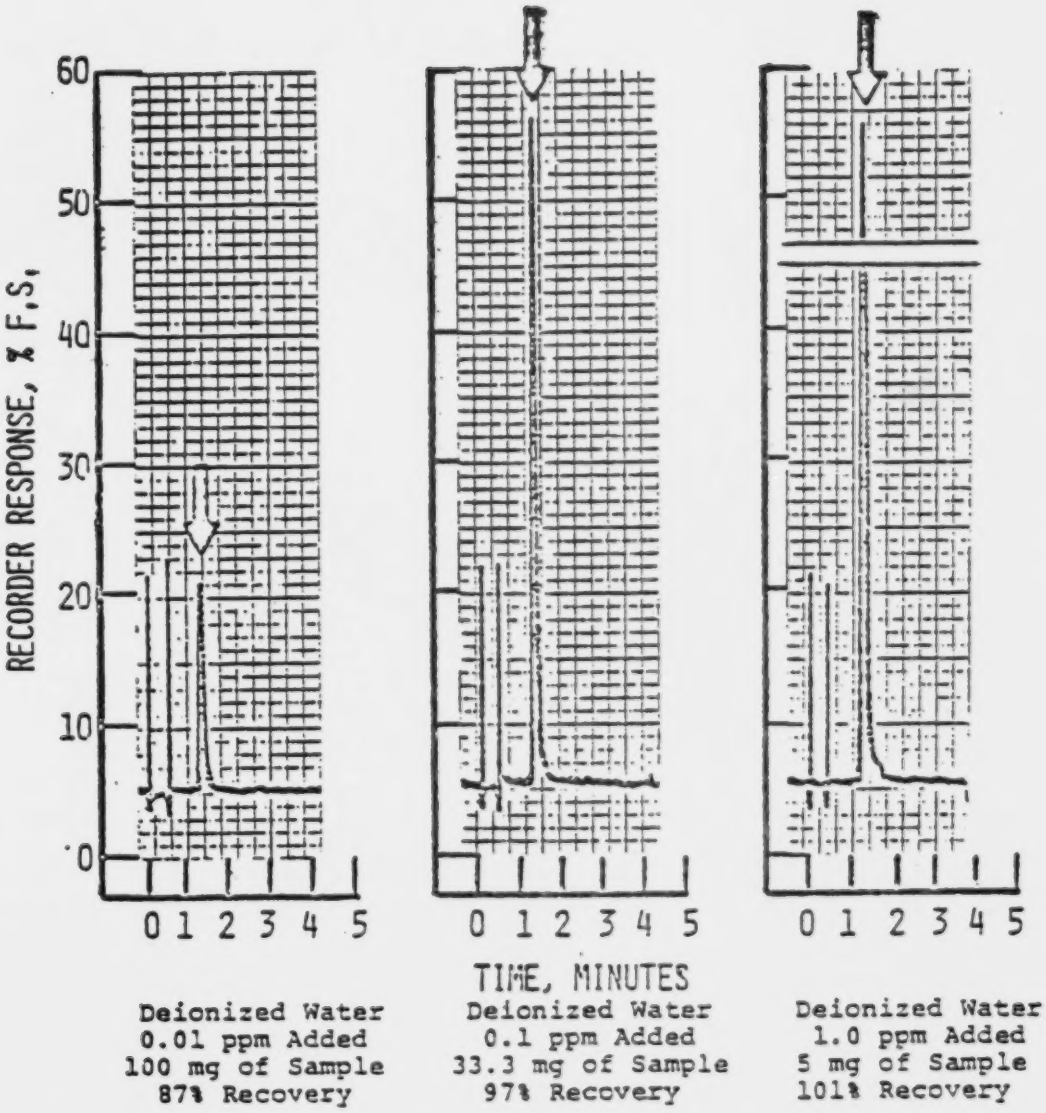


FIGURE 5. TYPICAL CHROMATOGRAMS OF WATER FORTIFIED WITH CHLORPYRIFOS.

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TEST METHOD

DETERMINATION OF COUMAPHOS IN WASTEWATER

METHOD 114

1. Scope and Application

1.1 This method covers the determination of coumaphos.

Parameter	Storet No.	CAS No.
Coumaphos	81293	56-72-4

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 A measured volume of water sample (250 mL) is extracted with chloroform. The extract is concentrated by rotary evaporation and analyzed by gas chromatography with alkali flame detector.

3. Interferences

3.1 All glassware used for analyzing wastewater samples must be pre-rinsed with chloroform and not previously used for any analysis other than wastewater. DO NOT USE PLASTIC WASH BOTTLES FOR ANY SOLVENTS.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Microsyringe, 10-uL

5.2.2 Rotoevaporator, Buchi or equivalent

5.3 Water Bath, 50-55°C

5.4 Gas chromatograph, Varian Model 1400 or equivalent, equipped with a phosphorus detector, an 18-in x 1/8-in O.D. glass column packed with 10% DC-200 (12,500 centistokes) on Gas Chrom Q 80/100 mesh, and glass injector and detector inserts. (If the ends of the column are long enough to be used as inserts, the glass inserts are not necessary.) Note: Use only Teflon-backed septums, Supelco No. 2-0459.

6. Reagents

6.1 Acetone, nanograde

6.2 Chloroform, nanograde

6.3 Hydrochloric acid, 1N -- Carefully add 21 mL of concentrated hydrochloric acid into a 250-mL glass-stoppered graduated cylinder containing approximately 200 mL of distilled water. Dilute to 250 mL with distilled water and mix thoroughly.

6.4 Mineral oil solution, 2.5% -- Dilute 25 mL of mineral oil, Fisher #0-120 or equivalent, to 1000 mL with nanograde chloroform, and mix thoroughly.

6.5 Sodium hydroxide, 1N -- Carefully dissolve 10 g of sodium hydroxide pellets, ACS, in 250 mL of distilled water and mix thoroughly.

6.6 Sodium sulfate, anhydrous, ACS

7. Calibration

7.1 Establish the GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedures

7.2.1 Coumaphos, standard solution, 0.10% -- Weigh 0.098-0.105 g of coumaphos (98% minimum purity) into a 100-mL volumetric flask. Dilute to volume with acetone, stopper, and mix thoroughly. This solution may be used for one week only.

7.2.2 Pipet a 1-mL aliquot of the 0.10% coumaphos solution into a 100-mL volumetric flask, dilute to volume with acetone, and mix thoroughly. Label this solution "10 ng". Prepare fresh daily.

7.2.3 Pipet 5-, 10- and 20-mL aliquots from the 10-ng standard of Step 7.2.2 into separate 100-mL volumetric flasks. Dilute to volume with acetone and mix. Label these solutions 500, 1000, and 2000 pg, respectively. Prepare fresh daily.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample by shaking, then proceed immediately to Step 10.2.

10.2 Measure 250 mL of the well-mixed sample into a 500-mL separatory funnel.

10.3 If the pH of the sample is above 7.0 or below 6.5, adjust it to 6.5-7.0 with 1N hydrochloric acid or 1N sodium hydroxide using pH indicator paper or a pH meter.

10.4 Extract three times by vigorously shaking for 1 minute each time with fresh 50-mL portions of nanograde chloroform. Collect the three chloroform extracts in another 250-mL separatory funnel. If an emulsion forms, centrifuge the emulsified layer and add the clear chloroform layer, obtained by centrifuging, to the second separatory funnel. Return the water layer to the first separatory funnel.

10.5 Add 50 mL of distilled water to the combined chloroform extracts in the second separatory funnel and shake for one minute.

10.6 Drain the chloroform layer through a funnel containing 4 to 5 g of anhydrous sodium sulfate retained on a small glass-wool plug (pre-rinsed with 10 mL of chloroform), into a 300-mL 24/40 boiling flask. Rinse the sodium sulfate three times with 10-mL portions of chloroform and add 10 mL of 2.5% mineral oil solution into the flask.

10.7 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.8 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.9 Pipet 5 mL of acetone into the flask from Step 10.8, stopper immediately, and rotate the flask so that the acetone washes down

the inside of the flask. DO NOT allow the acetone to get on the neck or stopper.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Continue the analysis according to TM A-54.12 (Mobay reference procedure).

13. Calculations

Not Available.

14. Method Performance

Not Available.

15. References

15.1 "Determination of Coumaphos in Wastewater by GLC," Analytical Method TM B-34.51, Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO.

Table 1

Chromatographic Conditions

	Varian 1400
Attenuation	X32
Cell voltage	Flame
Detector temperature, ^{o}C	210-230
Electrometer range	10^{-12}
Gas flows:	
Carrier gas	He, 30 mL/min Air, 230 mL/min H_2, 40 mL/min
Injector temperature	230-250°C
Oven temperature	230°C

Column: 18-in x 1/8-in O.D., glass, packed with 10% DC-200 (12,500 centistokes) on Gas Chrom Q 80/100 mesh.

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TEST METHOD

DETERMINATION OF CYANAZINE IN WASTEWATER

METHOD 115

1. Scope and Application

1.1 This method covers the determination of certain triazines. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Cyanazine	77780	21725-46-2
Simazine	39055	122-34-9
Propazine	39024	139-40-2

1.2 This is a thin-layer liquid chromatographic (TLC) method applicable to the determination of the compounds listed above in wastewater.

1.3 The method detection limit (MDL) is 1 mg/L for each parameter.

2. Summary of Method

2.1 A measured aliquot of water sample (50 mL) is extracted with chloroform. An aliquot of extract is used for spotting a TLC plate which is eluted with tetrahydrofuran/ethyl acetate/n-hexane. The plates are sprayed with $AgNO_3$ solution and developed by exposure to UV light.

3. Interferences

Not Available.

4. Safety

4.1 Plate spraying and exposure to UV radiation should be carried out

in a hood. Special glasses must be used when working with UV light. Eyes and skin must be protected from UV radiation.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Plate: Silica Gel G, neutral (50% Merck Silica Gel G 50% Bio Rad Bio Sil-A) on 200- x 200-mm glass plates coated at a thickness of 200 u.

5.2.2 Chamber: Supersaturated (filter paper lining)
Chamber size -- 25 cm x 29 cm x 10 cm.

5.2.3 Capillary tube from Kimax, size 1.6-1.8 x 100 mm.

6. Reagents

6.1 Chloroform

6.2 Cyanazine Standard

6.3 Simazine Standard

6.4 Propazine Standard

6.5 Eluent: Tetrahydrofuran/ethylacetate/n-hexane 4%/16%/80% (by volume)

7. Calibration

7.1 Normal principles of thin layer chromatography apply to this analysis. TLC conditions are summarized in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 0.0160 g each of propazine, simazine, and cyanazine of 99+% purity into a 100-mL volumetric flask. Dilute this stock standard to the mark with chloroform and carry out a serial dilution to the fifth dilution. These solutions are equivalent to 32, 16, 8, 4, 2, and 1 mg/L when compared to samples extracted 5:1.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Extract 50 mL of a water sample well with 10 mL of chloroform. Isolate the chloroform layer for spotting.

11. Cleanup and Separation

Not Available.

12. Thin Layer Chromatography

12.1 Table 1 summarizes the recommended operating conditions for TLC.

12.2 Calibrate the system as described in Section 7.

12.3 Perform one application of (7-8 uL) samples and standards using open-tip capillary tubes. Capillary tubes from Kimax size 1.6-1.8 x 100 mm.

12.4 After elution, air dry plate to remove solvents. Then spray with 0.02N $AgNO_3$ and expose to 1400-watt 366-nm wavelength ultraviolet light until maximum contrast is achieved. (DANGER -- PROTECT EYES AND SKIN FROM UV RADIATION.) Estimations are made by visual comparisons between samples and appropriate standards.

12.5 A fresh eluent should be prepared each day and should be allowed to equilibrate 1 hour before use.

12.6 Fresh standard solutions should be prepared once per month.

13. Calculation

Not Available.

14. Method Performance

14.1 A statement of "not detected" (ND) for a component indicates that the component is less than 1 mg/L (ppm).

14.2 The standard deviation of an analysis is $\pm$ 30% relative to actual concentration.

15. References

15.1 "TLC Determination of Propazine, Simazine, and Cyanazine in Wastewater," ETM-54-1, June 6, 1977, Ciba-Geigy, AL.

Table 1

Chromatographic Conditions

Temperature	Ambient
Distance	15 cm
Time	45 minutes
Eluent	Tetrahydrofuran/ethylacetate/n-hexane 4%/16%/80% (by volume)
Plate	Silica Gel G, neutral (50% Merck Silica Gel G 50% Bio Rad Bio Sil-A) on 200- x 200-mm glass plates coated at a thickness of 200 u.
Chamber	Supersaturated (filter paper lining) Chamber size -- 25 cm x 29 cm x 10 cm

Table 2

Typical Separation, Rf

Rf	Compound
0.3	Propazine
0.15	Simazine
0.1	Cyanazine
0.0	Point of origin

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TEST METHOD

DETERMINATION OF CYANAZINE [BLADEX(TM)] AND STIROFOS [TETRACHLORVINPHOS, RABON(TM)] IN WASTEWATER

METHOD 116

1. Scope and Application

1.1 This method covers the determination of cyanazine and stirofos. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Cyanazine (BLADEX)	77780	21725-46-2
Stirofos (Tetrachlorvinphos)(RABON)	—	961-11-5
Aldicarb	39053	16-06-3
Atrazine	39153	1912-24-9

1.2 This is a liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in aqeuous effluent samples.

1.3 The method detection limits (MDL) are 10 ug/L Aldicarb, 10 ug/L tetrachlorvinphos (RABON), 50 ug/L cyanazine (BLADEX), and 50 ug/L Atrazine.

2. Summary of Method

2.1 A measured volume of water sample (500 mL) is extracted with methylene chloride. The extract is concentrated to 0.5 mL and analyzed by liquid chromatography with UV detection.

3. Interferences

Not Available.

4. Safety

4.1 Aldicarb Acetone Solution is highly toxic in contact with the skin or eyes or if swallowed. Careful handling and special precautions are essential. Wear face shield, rubber gloves, rubber apron, and rubber footwear. If spillage occurs, flush promptly with copious amount of water. Destroy residual amounts of Aldicarb with 20-percent alcoholic sodium hydroxide. Do not eat during or after handling the material until after a thorough cleanup. Destroy all samples promptly after analysis by pouring them into a container of 20-percent alcoholic sodium hydroxide, which should be replenished daily.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.3 Liquid Chromatograph, Varian Aerograph Model 4010-01, equipped with ultraviolet photometric detector

5.3.1 Column, 25-cm x 1/8-in Micropak CN

5.3.2 Recorder, 1MV

6. Reagents

6.1 Chloroform, reagent grade

6.2 Aldicarb of known purity

6.3 Methylene chloride, pesticide grade

6.4 Heptane, reagent grade

6.5 Cyanazine (BLADEX) herbicide of known purity

6.6 Tetrachlorvinphos (BLADEX) insecticide of known purity

6.7 Atrazine of known purity

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 0.05 g Aldicarb, 0.02 g RABON, 0.10 g Cyanazine (BLADEX) and 0.1 g Atrazine into a 100 mL volumetric flask. Dilute to mark with methylene chloride. Dilute this

solution 1/10 in 10-mL volumetric flask. This solution contains 50 ug/mL Aldicarb, 20 ug/mL RABON, 100 ug/mL Cyanazine (BLADEX), and 100 ug/uL Atrazine.

7.2.2 The standards should be prepared fresh weekly. Sensitivity could easily be lowered tenfold by adjusting parameters of the method.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Extract 500 mL of sample according to procedure outlined in MLM 37 (Shell reference method).

10.2 Dilute the extract with 5 mL of methylene chloride and further concentrate by evaporating to 0.5 mL in a graduated centrifuge tube with a slow stream of air.

10.3 The chloroform/heptane should be vacuum stripped to remove the entrained gas before use.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

12.3 Measure the peak height of the RABON, Aldicarb, BLADEX, and Atrazine peaks and compare to the standard to determine the amount present.

13. Calculation

13.1 Calculate the amount of each component present as follows:

$$\text{ug/L (ppb)} = \frac{(\text{P.H.a}) \times (\text{ug/mL in std})}{(\text{P.H.b})}$$

Where: P.H.a = peak height of sample
P.H.b = peak height of standard

14. Method Performance

14.1 Method detection limits by this method are: Aldicarb - 10 ug/L, RABON - 10 ug/L, Cyanazine (BLADEX) - 50 ug/L, and Atrazine - 50 ug/L.

15. References

15.1 "Determination of Aldicarb Pesticide, RABON Insecticide, BLADEX Herbicide and ATRAZINE Herbicide in Aqueous Effluent, LC Method," Standard Test Method, Shell, Axis, AL.

Table 1

Chromatographic Conditions

Mobile Phase	65% Chloroform/35% Heptane
Pressure of Helium on mobile phase	750 psi
Flow of mobile phase	1 mL/min.
Sample size	10 uL
Range	0.04
Chart Speed	0.5 inches/min.
Column	25-cm x 1/8-in Micropak CN

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TEST METHOD

DETERMINATION OF 2,4-DB IN WASTEWATER

METHOD 117

1. Scope and Application

1.1 This method covers the determination of certain chlorophenoxy acids. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
2,4-DB	--	94-82-6
2,4-D	39730	94-75-7
MCPB	--	94-81-5
MCPA	--	94-74-6

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in treated process and area drainage water.

2. Summary of Method

2.1 A measured volume of water sample (100 mL) is extracted with ethyl ether and evaporated to near dryness. The extract residue is esterified and injected into a gas chromatograph with flame ionization detection.

3. Interferences

3.1 Chlorophenols, solvents, and alcohols are the impurities that would be extracted if present. Water-soluble organics such as glycolic acid do not extract and, in addition, also have short retention times. Isomers of phenoxyalkanoic acid will interfere with acids of other phenoxyacetic acids in some cases. If this occurs, different GLC columns will be needed to separate the isomers.

4. Safety

4.1 The use of ethyl ether is hazardous, as the material is highly flammable and the vapors are explosive. Evaporation is performed in a hood. Acetone is handled with care to prevent getting on hands (rubber gloves available when using) and inhaling vapors. Methanol esterification is performed in the hood. Steam baths are used instead of electrical heating to avoid sparking.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnels, 250 mL

5.2.2 Erlenmeyer flasks, 250 mL

5.2.3 Glass-stoppered graduates, 10 mL

5.2.4 Syringes with 4-inch needles, 1 mL

5.2.5 Centrifuge tubes, 15 mL

5.3 Hewlett Packard 5710A Gas Chromatograph with Flame Ionization Detectors

5.3.1 Autolab peak area Integrator

5.3.2 OV 101 3% on HMDS Chromosorb S, 1/8-in x 10-ft

6. Reagents

6.1 Standards - Recrystallized acid (3x) from benzene and methylene chloride

6.2 Sulfuric Acid (ACS) - 10% solution

6.3 Ethyl Ether (ACS)

6.4 0.1 N NaOH

6.5 Distilled water

6.6 Methanol - HCl esterification reagent. Prepared by adding Acetyl Chloride slowly to pesticide grade methanol.

6.7 Acetone (ACS)

6.8 Sodium Chloride (table salt grade)

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Make up standards by weighing 40 mg of the chlorophenoxy alkanoic acid in a glass weighing boat and transfer into a 1-liter volumetric flask. Use 5 - 10 mL acetone to rinse the glass boat into the flask. Add 200 mL of 0.1 N NaOH and warm on steam bath to dissolve acid(s). Cool and dilute to volume with 0.1 N NaOH. When 5 mL of this solution is diluted to 100 mL, the concentration is 2 ug/mL.

7.2.2 Add 95 mL of distilled water to a separatory funnel and 5 mL of the standard solution listed in Step 7.2.1. If area drainage water is to be analyzed, proceed to Step 7.2.3. If process water is to be analyzed, add approximately 5 g sodium chloride,shake until in solution, then continue with Step 7.2.3.

7.2.3 Add 5 mL of 10% H_2SO_4 solution and swirl to mix.

7.2.4 Extract with 35-, 20-, and 20-mL portions of ether. Any interface is left with the ether layer. Combine ether layers and interface wash twice with 10 mL of distilled water, leaving interface with ether, and save drainings. Pour ether extract into a 250-mL erlenmeyer flask from the top; interface should adhere to flask. Rinse top of separatory funnel with 2 - 3 mL of ether back into flask or into erlenmeyer. Add water wash drainings into separatory funnel and 10 mL of ether. Shake and drain water. Again pour ether from the top of separatory funnel into the erlenmeyer flask.

7.2.5 Put a boiling chip into the erlenmeyer and evaporate ether on a steam bath slowly. Use bath in a hood. Evaporate to 3 to 4 mL. Add enough salt to form a water-salt mass that will not flow. With a 1-mL syringe, transfer the ether layer to 15-mL centrifuge in a warm water bath. Add 2 - 3 mL of ether via the syringe to the flask rinsing down the sides. Swirl to break up salt mass and tip flask so that salt agglomerates in the one side; tip slowly in the opposite direction to drain ether. Transfer ether to 15-mL centrifuge tube. Repeat with another 1 mL of ether.

7.2.6 Evaporate contents of centrifuge tube to near dryness and add 5 mL of methanol-HCl reagent. Place on steam bath until HCl fumes cease to evolve (approximately 10 minutes).

7.2.7 After esterification is complete, transfer to 10-mL graduate with the 1-mL syringe using acetone to wash down

sides and rinsing tube. Use enough acetone to bring total to 10 mL in the graduate. Stopper and shake.

7.2.8 Inject 4 uL in the GLC at the conditions indicated. Integrate the peak area. Retention times of the acids are shown below:

Parameter	Seconds
MCPA	290
2,4-D	370
MCPB	655
2,4-DB	825

7.2.9 The uL injection size is equivalent to 0.08 ug of acid. Record data for calculations with sample.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Measure out 100 mL of well-stirred sample into separatory funnel (do not filter if there is any suspended material).

10.2 Add 5 mL of 10% H_2SO_4 solution, stopper, and shake. If CO_2 evolves when adding H_2SO_4, swirl without stopper until evolution ceases. Test solution with pH paper to make sure pH is 1 or less. Add more 10% H_2SO_4, if necessary.

10.3 Proceed as in Steps 7.2.4 through 7.2.8.

11. Cleanup and Separation

11.1 Cleanup methods (column type) are not needed as the effluents are fairly consistent and do not contain much organic matter that would interfere.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

13. Calculations

13.1 $\frac{\text{Sample Peak Area}}{\text{Standard Peak Area}} \times 2.0 = \text{mg/L phenoxyalkanoic acid in sample.}$

13.2 Calculation is based on the above 2.0 ug/mL (ppm) standards, solution volumes, and injection sizes. If different, make appropriate corrections.

14. Method Performance

14.1 The procedure is quantitative to 1 mg/L (ppm). Detectability is less than 0.5 mg/L (ppm).

15. References

15.1 "Determination of Chlorophenoxy Alkanoic Acids in Plant Effluent," Standard Test Method, Rhodia, Inc., Portland, OR.

Table 1

Chromatographic Conditions

Temperature	190°C
Flowrate	(Nitrogen) 60 mL/min
	(Hydrogen) 15 psig
Electrometer	Range 1, Attenuation 4
Column:	OV 101 3% on HMDS Chromosorb W, 1/8-in x 10-ft Single Channel Mode

Table 2

Retention Times

Parameter	Retention Time (seconds)
MCPA	290
2,4-D	370
MCPB	655
2,4-DB	825

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TEST METHOD

DETERMINATION OF DEET IN WASTEWATER

METHOD 118

1. Scope and Application

1.1 This method covers the determination of Deet.

Paramater	Storet No.	CAS No.
DEET	—	134-62-3

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 The sample is extracted with 15% methylene chloride in hexane. The extract is concentrated under a slow nitrogen stream to a final volume of 5 mL. Analysis is by GC-FID.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Wide-mouth glass jars such as the Mason type are recommended as suitable sample containers when the sample is to be 2 L or less. If the sample is of greater volume than 2 L, the 1-gallon glass bottles in which acetone, hexane, or petroleum ether are normally sold provide excellent sample containers. Furthermore, the latter require no special precleaning before use. The glass containers must be scrupulously cleaned and rinsed with some of

the same solvent used for subsequent pesticide extraction. All bottle or jar caps should be Teflon- or foil-lined to prevent contamination of the sample with trace quantities of impurities which may be present in laminated paper liners or in the composition of the material used for the seal in Mason jar lids.

5.2 Glassware and Other Equipment

5.2.1 Modified micro-Snyder Columns, 19/22, Kontes stock #K-569251.

5.2.2 Glass bead, 3-mm plain, Fisher #11-312 or equivalent.

5.2.3 Modified micro-Snyder column, 19/22 T joint, Kontes #K-569251.

5.2.4 Pipet, 4 mL.

5.2.5 Separatory funnels, 2 L with Teflon stopcocks.

5.2.6 Filter tubes, 150 x 24 mm, Corning 9480 or equivalent.

5.2.7 Kuderna-Danish concentrator fitted with graduated evaporative concentrator tube. These are available from the Kontes Glass Company, each component bearing the following stock numbers.

5.2.7.1 Flask, 500 mL, stock #K-570001

5.2.7.2 Snyder column 3-ball, stock #K503000

5.2.7.3 Steel springs, 1/2-in stock #K-662750

5.2.7.4 Concentrator tubes, 10 mL, Size 1025, stock #K580050

5.3 Water bath capable of maintaining 95° to 100°C.

5.4 Gas Chromatograph fitted with electron capture, flame photometric, and electrolytic conductivity detectors. GLC columns to be two of the three specified in Step 12.6, with all operating parameters as specified in Step 12.6.

5.4.1 Chromatographic columns, 22-mm I.D. x 300-mm length, with Teflon stopcock, without frits.

6. <u>Reagents</u>

6.1 Hexane, pesticide quality, distilled in glass.

6.2 Isooctane, pesticide quality.

6.3 Diethyl ether, AR grade, peroxide free. The ether must contain 2% (v/v) absolute ethanol. Most of the AR grade ethyl ether contains 2% ethanol, added as a stabilizer, and it is therefore

unnecessary to add ethanol unless it is found necessary to remove peroxides. The ether should be tested for the absence of peroxides by a suitable method.

6.4 Petroleum ether, pesticide quality, redistributed in glass, b.p. 30-60°C.

6.5 Methylene chloride, pesticide quality.

6.6 Methylene chloride/hexane, 15% v/v.

6.7 Eluting mixture (6%), 60 mL of diethyl ether is diluted to 1000 mL with petroleum ether, and approximately 15 g of anhydrous Na_2SO_4 is added to insure freedom from moisture.

6.8 Eluting mixture (15%), 150 mL of diethyl ether is diluted to 1000 mL with petroleum ether, and approximately 15 g anhydrous Na_2SO_4 is added.

6.9 Eluting mixture (50%), 500 mL of diethyl ether is diluted to 1000 mL with petroleum ether, and approximately 15 grams anhydrous Na_2SO_4 is added.

Note: None of the eluting mixtures should be held longer than 24 hours after mixing.

6.10 Anhydrous sodium sulfate, reagent grade, granular, Mallinkrodt stock #8024, or equivalent. Test for purity.

6.11 Florisil, 60/100 mesh, PR grade.

7. Calibration

Not Available.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 The sampling location and the method of drawing the sample will, to a great extent, be dictated by the objectives of the sample data. If the objective is to determine the highest pesticide pollution present in a stream or lake, a grab sample might be drawn at the point of highest pollution introduction. If, on the other hand, the objective is an average residue profile of the entire body of water, the final sample would preferably be a composite of a number of subsamples taken at various locations and water depths.

9.2 As implied by the name, a grab sample would be a surface water sample generally taken by simply filling the sample container by

immersing and allowing the bottle or jar to fill up. For sampling at selected depths, devices such as a Precision sewage water sampler or an Esmarch sampler may be utilized. Both devices consist of a metal outer container with a glass bottle inside as the sample collection vessel.

9.3 The Precision sampler, in which the interior of the collection bottle has free access to the exterior by means of an open tube, can be used to draw a composite depth sample. As soon as the device is immersed, collection of the sample is started. By premeasuring the rate of lowering the device to collect a given amount of water, an approximately uniform amount of water can be collected throughout the entire depth sampled.

9.4 The Esmarch sampler may be manually opened and closed by means of a chain attached to the bottle stopper. This permits a sample or subsample to be drawn from any given depth simply by lowering the device with the stopper closed, opening it at the proper sampling depth to permit filling of the collection bottle, then closing the stopper and raising the device to the surface.

9.5 The size of sample is dictated primarily by the expected residue levels. For example, if the sample is collected from a waterway where pesticide levels are expectedly high (such as agricultural run-off), a sample size of 500 to 1,000 mL may be sufficient. If the sample is drawn in connection with a monitoring program where no especially high residues would be expected, a sample size of 2 L or more may be indicated.

9.6 Ideally, analysis of the sample should be conducted within a matter of hours from the time of sampling. However, this is frequently impractical in terms of the distance from sampling site to laboratory, and/or the laboratory workload. Samples being examined solely for organochlorine residues may be held up to a week under refrigeration at 2° to $4^{\circ}C$. Those intended for organophosphorous or carbamate analysis should be frozen immediately after drawing sample and should be extracted no more than 4 days after sampling. These classes of pesticides undergo degradation rapidly in the aqueous medium.

9.7 Every effort should be made to perform the solvent extraction step at the earliest possible time after sampling, irrespective of the class of pesticides suspected as being present. The resulting extracts may then be held for periods of up to three or four weeks at -15° to $-20^{\circ}C$ before conducting the adsorbent partitioning and determinative portions of the analysis (see Table 1 of reference 15.5, not enclosed herein). These data show the degradation rate of 29 pesticides in water at ambient temperature in sealed containers.

9.8 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. <u>Sample Extraction</u>

10.1 Transfer 2 L of sample (or a lesser volume, if indicated) to a 4-L separatory funnel and add 120 mL of 15% methylene chloride/hexane.

Notes: 1. If, on the basis of prior analysis of a given waterway, the residue levels may be expected to run high, a sample of 500 mL or 1 L may be indicated. In this event, the size of the separatory funnel should be 2 L and the extraction solvent volumes given as 120 mL should be reduced to 100 mL.

2. A 500-mL graduated cylinder is a suitable measuring device for the initial sample. Any measuring discrepancy up to 5.0 mL would result in an error no greater than 1.0%.

10.2 Stopper funnel and shake vigorously 2 minutes. Allow layers to separate and draw off aqueous layer into a second 2 L separatory funnel.

10.3 Add another 120 mL of 15% methylene chloride/hexane to the aqueous phase in separatory funnel #2, stopper, and shake vigorously another 2 minutes.

10.4 Prepare a 2-inch column of anhydrous, granular Na_2SO_4 in a 150-x 24-mm filter tube with a small wad of pre-extracted glass-wool at the bottom. Position this over a 500-mL K-D flask to which is attached a 10-mL concentrator tube with one 3-mm glass bead in the bottom.

10.5 Filter the methylene chloride/hexane extract in separatory funnel #1 through the Na_2SO_4 column into the flask.

10.6 Draw off the aqueous layer in separatory funnel #2 into empty separatory funnel #1.

10.7 Add 120 mL of straight hexane to the aqueous solution in separatory funnel #1, stopper, and shake again for 2 minutes. Draw off and discard the aqueous layer.

10.8 Filter the solvent extracts in both separatory funnels through the Na_2SO_4 into the flask, rinsing down the filter tube with three 10-mL portions of hexane.

10.9 Attach a 3-ball Snyder column to the K-D flask, place assembly in a boiling water bath, and concentrate extract to approximately 5 mL.

10.10 Remove K-D assembly from bath, cool, and rinse ₹ joint between tube and flask with a small volume of hexane; also rinse down walls of tube. Rinse should be delivered with 2-mL Mohr pipet and should not exceed 3 mL.

10.11 Place tube under a slow nitrogen stream at ambient temperature and reduce extract volume to approximately 0.5 mL. Using a disposable pipet, carefuly add hexane to adjust volume to exactly 1.0 mL in the tube tip. Then, with a 4-mL pipet, add 4 mL of hexane. DO NOT rely on the accuracy of the tube graduation at the 5-mL mark.

10.12 Stopper concentrator tube and mix vigorously on Vortex mixer for 1 minute.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 The chromatographic approach should be made in accordance with the guidelines presented in Steps 12.6.1 through 12.6.6, and by applying the instrumental operating parameters specified for the respective GLC columns listed. By following the given protocol, it should be possible to make some tentative compound identification upon computation of relative retention times (RRT_A) of peaks in the preliminary chromatograms via electron capture.

12.2 Full reliance should not be placed on the chromatographic data obtained from one column. An alternate column of completely different compound elution characteristics should be used to (1) confirm a number of compounds tentatively identified on the first column, and (2) isolate and tentatively identify any compound pairs which may have eluted as single peaks on the first column. Subsequent quantitations are conducted as outlined in Section 12.6.

Note: If the initial chromatogram indicates the presence of a sufficient amount of interfering materials, it may prove necessary to conduct a Florisil cleanup on the extract. Based on the general experience of water chemists, this is rarely necessary on most surface water samples. If it should prove necessary, the cleanup should be carried out as prescribed in Section 5, A (1), pages 6-9 of Reference 15.5, not enclosed herein. After the cleanup, another exploratory injection is made followed by peak identifications and quantitation.

12.3 If the electron capture data indicate the probable presence of one or more chlorinated pesticide compounds, the chromatographer would be well advised to conduct confirmation via electrolytic conductivity detection in the reductive mode, even though positive identifications were made on two columns via electron capture. This extra step provides needed validation, particularly when compounds are tentatively identified which appear to be out of place in light of known supplemental data concerning the waterway sampled.

12.4 It is improbable that parent compounds in the organophosphorous class will be detected in an average water sample. Compound degradation is rather rapid in the aqueous medium. However, if the waterway received heavy run-off from nearby agricultural land undergoing current spray programs, the presence of these residuals is possible.

12.5 In general, many of the organophosphorous compounds are far less responsive to electron capture detection than compounds in the organochlorine glass. This factor, combined with their highly diluted concentration in a waterway, make electron capture detection a dubious matter. Therefore, specific detection is preferable for identification and quantitation of these compounds or their metabolites. Flame photometric detection provides a suitable mode for this mission. Guidelines for the use of this detector are provided in Step 12.6. It will undoubtedly be necessary to use the original 5-mL extract (undiluted) because of the expectedly low concentrations. In fact, it may prove necessary to concentrate the extract even further if a preliminary chromatogram via FPD gives any indication of peaks that may be of insufficient size to meet the response criteria given in Step 12.6.

12.6 Gas Chromatography-Electron Capture Columns

12.6.1 Column material shall be of borosilicate glass, 6-ft long, 1/4-in O.D., 5/32-in I.D. As off-column injection will be used, one side of the column shall be 1 inch longer than the other. The Swagelok nut, ferrule, and silicone "O" ring are assembled as in Section 4, A, (6) of Ref. 15.5, not enclosed herein. Complete column specifications for the Tracor MT-220 gas chromatograph are given in Section 4, A, (6) of Ref. 15.5, not enclosed herein.

12.6.2 Column Selection -- There is a wide variety of column packing materials in the marketplace, some of which are entirely suitable for use in pesticide analysis, and others which are of limited value. In general, the columns selected as a "working pair" should be significantly different in polarity and in their compound elution characteristics. One pair that has proved very useful is given as A and C of Section 4, A, (6) of Ref. 15.5, not enclosed herein. B provides another alternate. The peak elution patterns for 13 chlorinated pesticidal compounds on each of these columns are shown in Figures 1 through 3 of Section 4, A, (6) of Ref. 15.5, not enclosed herein.

12.6.2.1 1.5% OV-17/1.95% OV -210 -- Liquid phases premixed and coated on silanized support, 80/100 mesh.

12.6.2.2 4% SE-30/6% OV-210 -- Liquid phases premixed and coated on silanized support, 80/100 mesh.

12.6.2.3 5% OV-210 -- coated on silanized support, 100/120 mesh.

12.6.3 Packing the Column:

12.6.3.1 Make certain the column is actually 6 ft long. A paper template tacked to the wall is a convenient and quick means of checking. For off-column injection in the Micro-Tek Model 220, one column leg should be 1 inch shorter than the other.

12.6.3.2 With a china marking pencil, place a mark on the long column leg 2 in from the end. Place a similar mark 1-1/8 inch from the end of the short leg.

12.6.3.3 Add the packing to the column through a small funnel, approximately 6 in at a time, and bounce the column repeatedly on a semihard surface. Rapid tapping up and down the column with a wooden pencil will promote settling of the packing. The packing is added until it reaches the mark on each leg and it is found that additional tapping will not produce any further settling.

Note: This operation should be done with great care, tapping the column a sufficient length of time to be <u>certain</u> that no further settling is possible by manual vibration. The use of mechanical vibrators is not advised as the packing can be packed too densely, thus introducing the possibility of an excessive pressure drop when carrier gas is applied.

12.6.3.4 Pack silanized glass wool into both ends of the column just tightly enough to prevent dislodging the carrier flow. The glass wool should completely fill the space from the top of the packing to the end of the column.

Note: If the glass wool is manipulated by hand, the hands should be carefully prewashed with soap or detergent, rinsed and dried. This minimizes the possibility of skin oil contamination of the glass wool.

12.6.4 Column Conditioning -- The column is conditioned, or made ready for use, in two operations: (1) by heat curing, and (2) by silylation treatment.

12.6.4.1 Heat Curing

12.6.4.1.1 A Swagelok fitting is attached to the inlet port at the top of the oven. This is comprised of a 1/4-inch Swagelok to AN adapter, part number 400-A-4ANF, connected to a 1/4-inch male union, part number 400-6.

12.6.4.1.2 Before assembling, the bore of the union must be drilled out with a 1/4-in drill and burnished with a rat-tailed file so that it will accept the 1/4-in O.D. column glass.

12.6.4.1.3 The short column leg is attached to the fitting, with the end of the long leg venting inside the oven. The nut, ferrule, and "O" ring are assembled as shown in Fig. 4 of Section 4, A, (6) of Ref. 15.5, not enclosed herein. Make sure the nut is tight, as the "O" ring will shrink during the curing period, thus allowing carrier gas to escape.

Note: The outset ports leading to the transfer line should be sealed off during the conditioning period to prevent traces of column effluent from seeping through to the detector. This is easily done by assembling a 1/4-inch Swagelok nut on a short piece of 6-mm glass rod with ferrule and "O" ring.

12.6.4.2 Silylating Treatment

12.6.4.2.1 Treatment with a silylating compound such as Silyl 8 serves to block active absorption sites, particularly prevalent in a new column, thereby somewhat improving efficiency and resolution characteristics. The most drastic effect is in the improvement of endrin response and the near elimination of on-column breakdown of endrin. Silyl 8 is available in 1- and 25-mL septum capped bottles from the Pierce Chemical Company, P.O. Box 117, Rockford, IL 61105.

12.6.4.2.2 At the end of the prescribed heat curing period, adjust the oven tempset and carrier gas flow controllers to the appropriate settings to give the

approximate recommended operating parameters for the given column. While the temperature is dropping, open the oven door and, wearing heavy gloves, retighten the Swagelok nut which will invariably loosen during heat curing. Close the door and allow oven temperature to equilibrate. Make four consecutive injections of 25 uL each of Silyl 8, spacing the injections approximately 1/2 hour apart. Allow at least three hours for the final injection to elute off the column before proceeding.

Notes: Syringe used for Silyl 8 injection should be used for no other purpose and should be flushed with benzene immediately after use to avoid plugging of the needle.

It is strongly advised that Silyl 8 be discarded after one year and that fresh material be ordered; some troublesome side effects have been noted in electon capture GLC arising from the use of old Silyl 8.

13. Calculation

Not Available.

14. Confirmation

14.1 It is assumed that final TLC and electrolytic conductivity confirmation may be applied to supplement the information obtained by electron capture detection. For this reason a larger sample is used than would be necessary for electron capture alone. Dilution of an aliquot of the final extract for electron capture -- GLC requires far less time than the extraction of another sample for confirmatory purposes.

15. References

15.1 FEDERAL REGISTER, Vol 38, No. 125, 29 June 1973, Part II.

15.2 Manual of "Method for Organic Pesticides in Water and Wastewater," 1971, EPA, NERC, Cincinnati, OH.

15.3 Eichelberger, J.W. and Lichtenberg, J.J., Persistence of Pesticides in River Water, Envir. Sci. & Technol., 5, No. 6, June 1971 (Table 1).

15.4 "The Sampling and Analysis of Water for Pesticides," Standard Test Method, Hardwicke Chemical Company, Elgin, SC.

15.5 Manual of "Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples," Dec. 1974, U.S. Environmental Protection Agency, Environmental Toxicology Division, Research Triangle Park, NC 27711.

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United States
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Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF MEVINPHOS [PHOSDRIN(TM)], DICHLORVOS [VAPONA(TM)], NALED [DIBROM(TM)], AND STIROFOS [RABON(TM)] IN WASTEWATER

METHOD 119

1. Scope and Application

1.1 This method covers the determination of certain organophosphate pesticides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Stirofos [RABON(TM)]	--	961-11-5
Naled [DIBROM(TM)]	--	300-76-5
Mevinphos [PHOSDRIN(TM)]	39610	7786-34-7
Dichlorvos [VAPONA(TM)]	--	62-73-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in wastewater.

1.3 The method detection limits (MDL) are listed in Table 1.

2. Summary of Method

2.1 A measured volume of water sample (100 mL) is passed through a C_{18}-Sep Pak. The Sep Pak is subsequently eluted with ether and analyzed after concentration to 1 mL. An optional cleanup is effected by diluting the ether eluent with hexane and passing it through a Silica Sep Pak, followed by ether elution and concentration. Analysis is by gas chromatography with flame ionization detector.

2.2 This method provides an optional cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 All glassware must be scrupulously clean to avoid contamination.

4. Safety

4.1 Ether, acetone, and hexane are extremely flammable. Use in well ventilated area. Keep away from all flames and sources of ignition. Use methylene chloride in a well ventilated area.

4.2 VAPONA, PHOSDRIN, and DIBROM are extremely toxic insecticides. Extreme care must be used when handling these insecticides. See Material Safety Data Sheet for handling instructions, not enclosed herein. Impervious gloves must be worn.

4.3 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Class A pipets, 1 mL and 10 mL

5.2.2 Volumetric flasks, 100 mL and 1 L

5.2.3 Centrifuge tubes (10 mL) graduated in 0.1-mL increments

5.2.4 Glass syringes, 5, 10 and 100 mL

5.2.5 Hamilton syringe, 10 uL

5.3 C_{18} and Silica Sep-Pak cartridges

5.4 Gas Chromatograph, Varian 3700 or equivalent, equipped with a flame photometric detector with phosphorous filter

5.4.1 Chromatographic column, 1/4-in x 6-ft glass tubing packed with 1:1 mixture of 3% OV-225 on 100/120 Gas Chrom Q and 3% QF-1 on 80/100 Gas Chrom Q, or equivalent.

5.4.2 Data System, Sigma 10, or suitable equivalent or strip chart recorder.

6. Reagents

6.1 Ether, reagent grade, ACS

6.2 Hexane, distilled in glass, suitable for gas chromatography and residue analysis

6.3 Sodium sulfate, anhydrous, reagent ACS

6.4 Dichlorvos (VAPONA Insecticide), analytical standard of known purity

6.5 Naled (DIBROM Insecticide), analytical standard of known purity

6.6 Stirofos (RABON Insecticide), analytical standard of known purity

6.7 Mevinphos (PHOSDRIN Insecticide), analytical standard of known purity

6.8 Acetone, distilled in glass, suitable for gas chromatography and residue analysis

6.9 Methylene chloride, distilled in glass, suitable for gas chromatography and residue analysis

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 2.

7.2 Calibration Procedure

7.2.1 Standard Stock Solution: Weigh to the nearest 0.1 mg, approximately 0.5 g each of VAPONA, PHOSDRIN, DIBROM, AND RABON into a 100-mL volumetric flask. Dilute to the mark with methylene chloride and mix well. Note: If other pesticides are not to be analyzed by liquid chromatography, acetone may be used as the solvent in this step. Correct for the purity and calculate the concentration of each component in mg/mL. (This solution will contain approximately 5 mg/mL of each insecticide.)

7.2.2 Intermediate Standard Solution: Pipet 1.0 mL of the Standard Stock Solution into a 100-mL volumetric flask. Dilute to the mark with hexane and mix well. Calculate the concentration of each component in mg/mL. (This solution will contain approximately 0.05 mg/mL of each insecticide.)

7.2.3 Working Standard Solution: Pipet 10.0 mL of the Intermediate Standard Solution into a 100-mL volumetric flask. Dilute to the mark with hexane and mix well. Calculate the concentation of each component in ug/mL to the nearest 0.2 ug/mL. (This solution will contain approximately 5 ug/mL of each insecticide.)

7.2.4 Inject 5 uL of the VAPONA, PHOSDRIN, DIBROM, and RABON Working Standard Solution onto the gas chromatograph under the chromatographic conditions shown in Table 2.

Note: All standard solutions should be prepared fresh monthly.

7.2.5 Allow the chromatogram to develop. Figure 1 is a typical chromatogram.

7.2.6 If a reporting integrator is used, calibrate the integrator for each component by entering 10 times its actual ug/mL value determined in Step 7.2.3. Example: 5.3 ug/mL x 10 = 53. This corrects for concentrating the sample 100 times, and the reporting integrator will now report values in actual ug/L (ppb) concentration.

7.2.7 Inject 5 uL of the 1.0-mL ether concentrate and allow chromatogram to develop.

8. Quality Control

8.1 Spike Stock Solution: Weigh, to the nearest 0.1 mg, approximately 0.5 g each of VAPONA, PHOSDRIN, DIBROM, and RABON into a 100-mL volumetric flask. Dilute to the mark with acetone. Correct the concentration for purity.

8.2 Spike Working Solution: Pipet 1.0 mL of the Spike Stock Solution into a 100-mL volumetric flask and dilute to the mark with acetone.

8.3 Spiked Sample: Pipet 1.0 mL of the Spike Working Solution into a 1-liter volumetric flask and dilute to the mark wtih the sample. This spiked sample contains approximately 50 mg/mL of each spike and should be handled exactly as the unspiked sample.

8.4 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Clean each C_{18} Sep Pak by eluting 10 mL of reagent grade acetone through the Sep Pak and then follow with 20 mL of water to activate the Sep Pak.

10.2 Attach the C_{18} Sep Pak to a 100-mL graduated glass syringe. Pour 100 mL of sample into the syringe. Attach to a vacuum and slowly pull the sample through the C_{18} Sep Pak. Discard the eluate. Place the C_{18} Sep Pak on vacuum for 5 to 10 minutes to remove as much water as possible.

Note: If the sample is extremely "dirty," use the Silica Sep Pak for further clean-up using the procedure in Section 11. (This procedure is usually not necessary.) Otherwise continue with Section 10.3.

10.3 Elute off the pesticides with 5 mL of ether into a 10 mL-graduated centrifuge tube.

10.4 Using a heating block, concentrate the 5 mL of ether eluate to 1.0 mL with low heat and a gentle stream of air. The original sample has now been concentrated 100 times and is ready for analysis by GC.

11. Cleanup and Separation

11.1 Clean each Silica Sep Pak by eluting 10 mL of reagent grade ether through the Sep Pak and then follow with 20 mL of reagent grade dry hexane to activate the Sep Pak.

11.2 Using the C_{18} Sep Pak prepared in Step 10.2 which contains the adsorbed sample, elute off the pesticides with 5 mL of ether into a 100-mL stoppered graduated cylinder.

11.3 Add approximately 95 mL of dry hexane and 2 to 3 g of Na_2SO_4. Shake well.

11.4 Attach the Silica Sep Pak to a 100-mL graduated glass syringe and pour the 100-mL hexane/ether solution into the syringe. Attach to vacuum and slowly pull the sample through. Discard the eluate.

11.5 Continue as written in Step 10.3.

12. Gas Chromatography

12.1 Table 2 summarizes the recommended operating conditions for the liquid chromatograph. Included in Table 1 are the estimated retention times and method detection limits.

12.2 Calibrate the system as described in Section 7.

13. Calculations

13.1 If a reporting integrator is used, ug/L (ppb) concentration may be read directly from the report.

If only a strip recorder is available and peaks are well chromatographed, measure peak heights (PH) to the nearest millimeter and calculate as below:

pesticide, ng/mL =

$$\frac{\text{PH of component in sample}}{\text{PH of component in std}} \times \frac{\text{ug of component in std}}{\text{mL}} \times \frac{1{,}000\ \text{ng}}{1\ \text{ug}} \times \frac{1\ \text{mL}}{100\ \text{mL}}$$

or

pesticide, ug/L (ppb) =

$$\frac{\text{PH of component in sample}}{\text{PH of component in std}} \times 10 \times \text{ug/mL of component in std}$$

14. Method Performance

14.1 Detection limits by this method are:

PHOSDRIN - 2 ug/L, VAPONA - 2 ug/L, DIBROM - 10 ug/L, and RABON - 2 ug/L.

14.2 Other ranges of sensitivity may be determined by this method by varying concentrations of standards and instrument parameters.

14.3 Limited accuracy and precision data are available for this method.

Plant effluent was spiked and analyzed on ten different days to indicate method accuracy. Results are presented in Table 3.

14.4 Low recovery of VAPONA is expected because of hydrolysis. Low recovery of DIBROM can be attributed to both hydrolysis and thermal degradation in the injection port.

Plant effluent was spiked and analyzed on three different days in duplicate by two technicians to indicate method precision. Results are presented in Table 3.

14.5 Generally, duplicate analyses should agree within 15% of the mean value.

15. References

15.1 "Determination of Phosdrin(R), Vapona(R), Dibrom(R), and Rabon(R) Insecticides in Aqueous Effluent, Gas Chromatograph (GC) - Flame Photometric Detector (FPD) Method," Standard Test Method, Shell Chemical Company, Axis, AL.

Table 1

Retention Times and Detection Lmits

Parameter	Retention Time (min)	Detection Limit (ug/L)
stirofos	12.5	2
naled	7.65	10
mevinphos	6.02	2
dichlorvos	1.84	2

Table 2

Chromatographic Conditions

Injection Port Temperature	150°
Initial Oven Temperature, Time	$120^{\circ}C$, 3 min.
Final Temperature, Rate, Time	$230^{\circ}C$, $10^{\circ}C$/min, 10 min.
Detector Temperature	$250^{\circ}C$
Hydrogen Flow Rate	140 mL/min
Air #1 Flow Rate	80 mL/min
Air #2 Flow Rate	170 mL/min
Helium Flow Rate	70 mL/min
Range	10^{-9}
Attenuation	8
Recorder Speed	0.5 cm/min
Recorder Attenuation	as required for optimum signal

Column: 1/4-in x 6-ft glass packed with 1:1 mixture of 3% OV-225 on 100/120 Gas Chrom Q and 3% QF-1 on 80/100 Gas Chrom Q.

Table 3

Method Accuracy

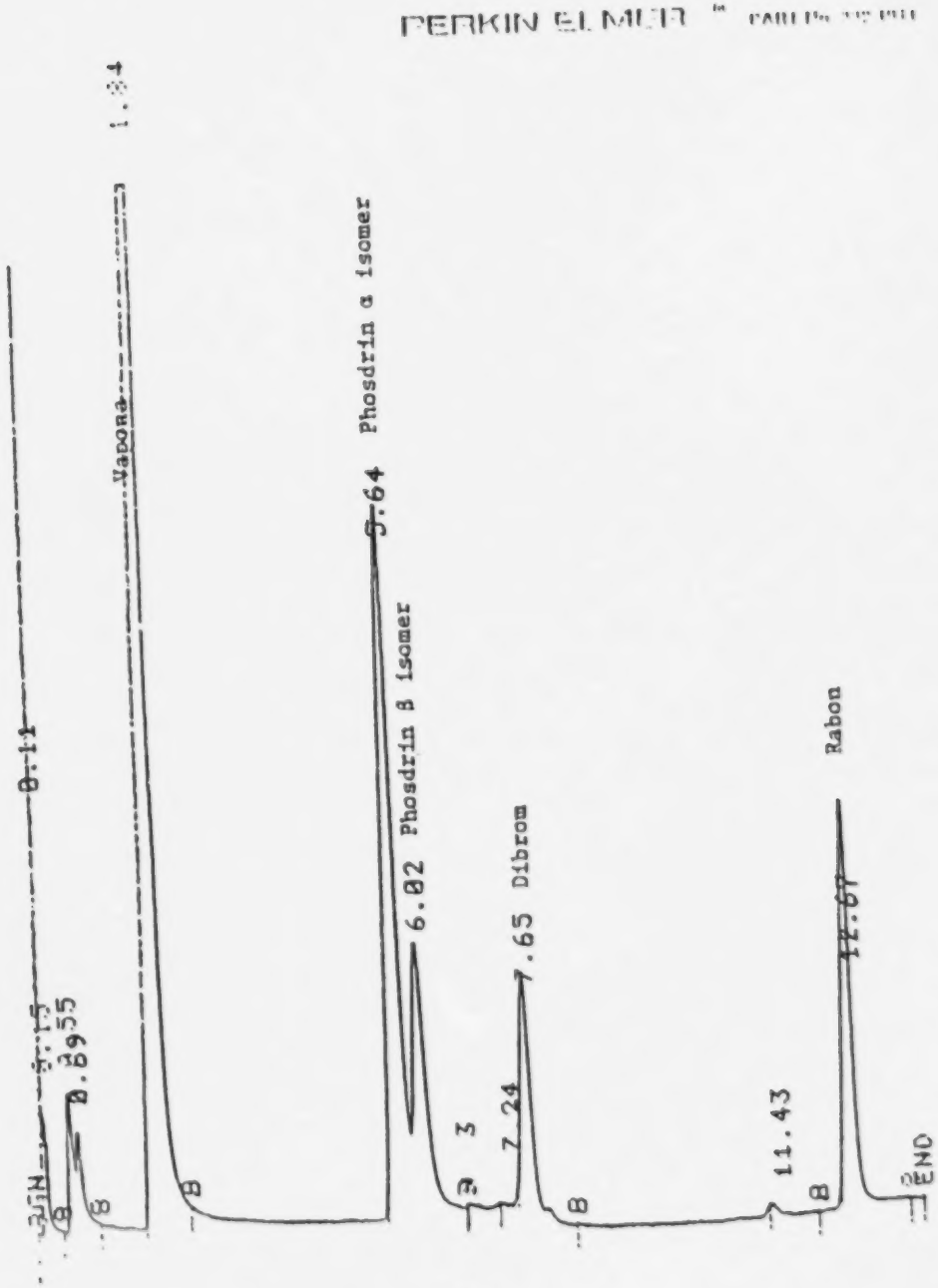
Pesticide	Spike ug/L	Mean Recovery %	Standard Deviation
dichlorvos	54	79.0	7.3
mevinphos	50	93.7	4.9
naled	49	40.5	15.0
stirophos	50	92.4	8.5

Method Precision

	ug/L					
	Sample #1 Determination		Sample #2 Determination		Sample #3 Determination	
Pesticide	#1	#2	#1	#2	#1	#2
dichlorvos	51	49	47	51	48	54
mevinphos	36	41	53	53	49	50
naled	11	11	<10	<10	12	14
stirophos	15	15	42	48	39	43

FIGURE 1

PESTICIDE STANDARD



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Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF MEVINPHOS [PHOSDRIN(TM)], DICHLORVOS [VAPONA(TM)], NALED [DIBROM(TM)], AND STIROFOS [GARDONA(TM)] IN WASTEWATER

METHOD 120

1. Scope and Application

1.1 This method covers the determination of certain organophosphorus pesticides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Dichlorvos [VAPONA(TM)]	--	62-73-7
Mevinphos [PHOSDRIN(TM)]	39610	7786-34-7
Naled [DIBROM(TM)]	--	300-76-5
Stirofos [GARDONA(TM)]	--	961-11-5

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in aqueous effluent.

1.3 The method detection limits (MDL) for many of the parameters are listed in Table 1.

2. Summary of Method

2.1 The organic extractables are dissolved in 5 mL of methylene chloride. The sample is concentrated by reducing the volume to 0.5 mL by use of a slow stream of air.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Hamilton 10-uL syringe.

5.3 Gas chromatographic, Tracor Model 550 or equivalent, equipped with a flame photometric detector with phosphorous filter.

5.3.1 Chromatographic column, 1/4-in x 4-ft glass tubing packed with 3% QF-1 on Chromosorb W, AW-DMCS treated.

6. Reagents

6.1 Methylene Chloride, Reagent grade

6.2 Dichlorvos (VAPONA) Insecticide, known quality

6.3 Naled (DIBROM) Insecticide, known quality

6.4 Stirophos (GARDONA) Insecticide, known quality

6.5 Mevinphos (PHOSDRIN) Insecticide, known quality

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 2.

7.2 Calibration Procedure

7.2.1 Weigh 0.025 g Dichlorvos (VAPONA), 0.020 g Stirofos (GARDONA), 0.50 g Naled (DIBROM), and 0.20 g Mevinphos (PHOSDRIN) into a 10-mL volumetric flask and dilute to the mark with methylene chloride.

7.2.2 Pipet 0.1 mL of this stock solution into another 10-mL volumetric flask and dilute to the mark with methylene chloride. This solution contains 25 ug/mL Dichlorvos, 20 ug/mL Stirofos, 500 ug/mL Naled, and 200 ug/mL Mevinphos.

7.2.3 Inject 2 uL of the standard solution onto the gas chromatograph column.

7.2.4 Determine peak height in centimeters of the standards.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 After determining the organic extractables as outlined in MLM 37 (Shell reference method), dissolve the extractables in 5 mL of methylene chloride. Further concentrate the sample by reducing the volume to 0.5 mL in a graduated centrifuge tube by use of a slow stream of air.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 2 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Inject 2 uL of the concentrate onto the gas chromatograph column.

12.4 Measure peak height in centimeters of components in sample.

13. Calculations

13.1 Calculate the amount of each insecticide present as follows:

Insecticide, ug/L =

$$\frac{\text{(Peak height of component in sample)(ug/mL component in standard)}}{\text{(Peak height of component in Standard)}}$$

14. Method Performance

14.1 Other ranges of sensitivity may be determined by this method by varying concentrations of standards and instrument parameters.

14.2 Detection Limits by this method are : PHOSDRIN-100 ug/L, VAPONA-10 ug/L, DIBROM-100 ug/L, and GARDONA-10 ug/L.

15. References

15.1 "Determination of Phosdrin(R), Vapona(R), Dibrom(R), and Gardona(R) Insecticides in Aqueous Effluents, GC-FPD Method," Standard Test Method, Shell Co., Axis, AL.

Table 1

Detection Limits

Parameter	Method Detection Limit (ug/L)
Dichlorvos (VAPONA)	10
Mevinphos (PHOSDRIN)	100
Naled (DIBROM)	100
Stirophos (Tetrachlorvinphos)(GARDONA)	10

Table 2

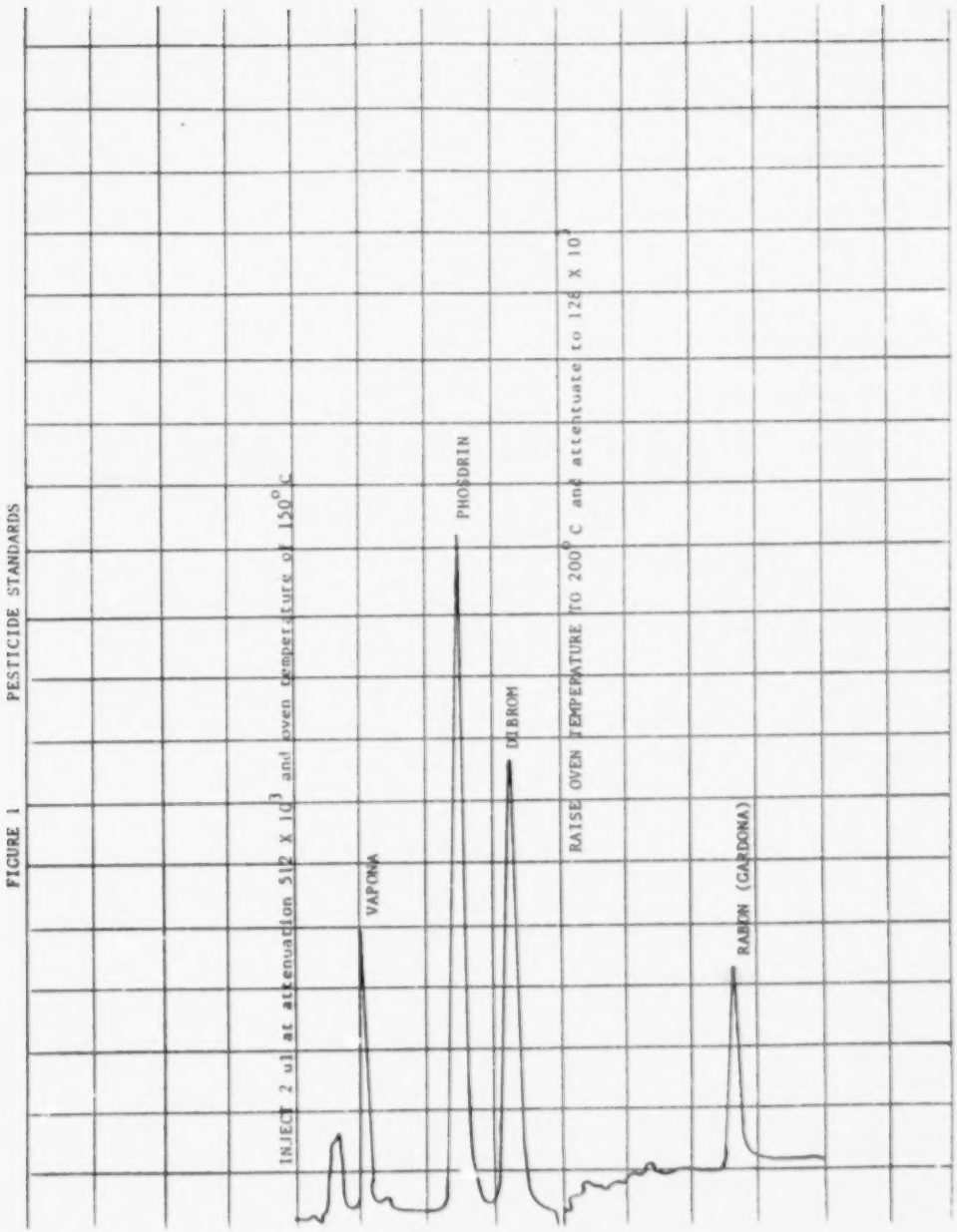
Chromatographic Conditions

Injection Port Temperature	250°
Oven Temperature	$140^{\circ}C$ - $200^{\circ}C$ (after elution of DIBROM)
Detector Temperature	$250^{\circ}C$
Hydrogen Flow Rate	28 mL/min
Nitrogen Flow Rate	70 mL/min
Air Flow Rate	180 mL/min
Attenuation	32×10^{3}
Recorder Speed	0.5 in/min

Column: 1/4-in x 4-ft glass packed with 3% QB-1 on Chromosorb W, AW-DMCS treated.

FIGURE 1

PESTICIDES STANDARDS



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Water and Waste Management

TEST METHOD

DETERMINATION OF DINOSEB (2-SEC-BUTYL-4,6-DINITROPHENOL) IN WASTEWATER

METHOD 121

1. Scope and Application

1.1 This method covers the determination of dinoseb (DNBP).

Parameter	Storet No.	CAS No.
Dinoseb	—	88-85-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in water.

2. Summary of Method

2.1 Acidified wastewater samples are extracted with diethyl ether, and the extract is cleaned up on alumina. The aqueous eluate from the alumina column is re-acidified and re-extracted with diethyl ether before derivatization with diazomethane. The derivatized extract is evaporated to dryness, dissolved in 1 mL of hexane, and cleaned up on an acidic alumina column. The column is eluted with diethyl ether, and the eluate is analyzed by GC with electron capture detector.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

Not Available.

4. Safety

4.1 Diazomethane is toxic, can cause specific sensitivity, and is potentially explosive. Do not breathe vapor or expose to skin or eyes. Preparation of diazomethane reagent should be

performed in a hood. Ground glass joints and sharp edges should be avoided. Thus, all connections should be made with rubber stoppers, glass tubing should be fire polished, and etched or scratched glassware should not be used. Diazomethane solutions should not be exposed to direct sunlight or strong artificial light. (Ref. 15.1)

4.2 Benzene is flammable. LD_{50} orally in rats is 5.7 g/kg.

Methanol is flammable. Poisoning in humans may occur from ingestion, inhalation, or percutaneous absorption. Diethyl ether is flammable. Chloroform is toxic. These reagents should be used in well ventilated areas away from an ignition source.

4.3 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Syringe - 10 mL (Hamilton No. 701N)

5.2.2 Capsule vials, screw cap, No. 4 - 4-1/4 in, Kimble No. 60957, 40-mL capacity

5.2.3 Graduated cylinder, glass-stoppered, 100 mL

5.2.4 Borosilicate glass column; about 1 x 14 cm, fitted with coarse fritted disc

5.2.5 Volumetric flask, 10 mL

5.2.6 4 oz., square, amber bottles

5.3 Filter-cel filter-aid, Johns-Manville Corporation

5.4 Mechanical shaker

5.5 Clinical centrifuge

5.6 Lourdes Model MM-1 Multi-Mix Homogenizer.

5.7 Rotary evaporator

5.8 Gas chromatograph system equipped with an electron capture detector. A Barber-Colman Model 5000 gas chromatograph.

5.8.1 Gas chromatograph column; U-shaped borosilicate glass, 74-in x 3-mm I.D.

5.8.2 Barber-Colman Sr^{90} ionization detector, Model A-4150. Operating voltage for the detector was derived from a 90-volt dry-cell battery connected in parallel with a 10-turn 300-K potentiometer. A 2000-uF capacitor was connected between the anode of the detector cell and chassis ground to reduce baseline noise.

6. Reagents

6.1 Benzene, glass distilled

6.2 Methanol, glass distilled

6.3 Chloroform, ACS

6.4 Diethyl ether, Analytical Reagent

6.5 2-sec-butyl-4,6-dinitrophenol (DNBP), analytical grade; obtainable from Agricultural Department, The Dow Chemical Company, Midland, MI

6.6 2-sec-butyl-4,6-dinitrophenol, methyl ether (DNME); obtainable from Sampling Coordinator, Agricultural Department, The Dow Chemical Company, Midland, MI

6.7 Dow Corning 200 fluid 12,000 cps. Dow Corning Corporation, Midland, MI

6.8 Gas-Chrom Z, solid support, 80-100 mesh, Applied Science Laboratories, Inc., 140 North Barnard Street, State College, PA

6.9 Alumina; Woelm basic and acidic alumina, (kept at 130°C); Alupharm Chemicals, P.O. Box 30628, New Orleans, LA 70130

6.10 Phosphoric acid, 85%

6.11 Sodium chloride, ACS

6.12 Sulfuric acid, 6 N

6.13 Prepurified nitrogen (carrier gas), Air Reduction Co.

6.14 2-(2-Ethoxyethoxy)ethanol, J.T. Baker Chemical Co., Phillipsburg, NJ

6.15 Diazald N-methl-N-nitroso-p-toluenesulfonamide, Aldrich Chemical Company, Inc., 2369 North 29th Street, Milwaukee, WI

6.16 Pipets, disposable, capillary, 5-3/4 inch long. Scientific Products #P5205-2.

6.17 Diazomethane solution (2): Approximately 18 mg per mL in ether. Add 35 mL of 2-(ethoxy-ethoxy)ethanol and 10 mL of ether to a solution of 6 g of KOH in 10 mL of distilled water contained in

a 100-mL long-necked distilling flask. Place a magnetic stirring bar in the flask and mount in a water bath on top of a hot plate magnetic stirrer. Attach a dropping funnel and an efficient condenser connected to two receiving flasks in series. A 350-mL Erlenmeyer serves as the first flask, followed by a 50-mL Erlenmeyer flask containing 25 mL of ether. The inlet tube of the second receiver should dip below the surface of the ether. Cool both receivers to 0°C. Place a solution of 21.5 g of Diazald reagent in 140 mL of ether in the dropping funnel. While stirring the contents of the flask, heat the water to 65°C. As the distillation of ether begins, add the Diazald over a 20-minute period. Follow with 20 mL of ether and interrupt the distillation when the distillate appearing in the condenser is nearly colorless. Combine the contents of the two receivers and store in culture tubes with foil-lined screw caps. The reagent is stable for several weeks if kept below 0°C, in full, closed tubes.

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Dissolve 0.053 g 2-sec-butyl-4,6-dinitrophenol, methyl ether (DNME) in 500 mL of benzene to give a solution equivalent to 100 ug/mL DNBP. Make appropriate volumetric dilutions of this solution with glass-distilled benzene to obtain standard solutions covering the concentration range from 0.01 to 0.05 ug/mL.

7.2.2 Inject 4 uL aliquots of DNME standard solutions, covering the concentration range from 0.01 to 0.05 ug/mL into the chromatograph and record the resulting peak heights. Plot peak heights on the ordinate as percent full-scale deflection vs. corresponding concentrations of DNME on the abscissa.

8. Quality Control

8.1 Prepare 2-sec-butyl-4,6-dinitrophenol (DNBP) methanolic spiking solutions (spiking standard). Dissolve 0.050 g of DNBP in 500 mL of benzene to obtain a 100-ug/mL solution. Dilute 1, 3, 5, and 10 mL of benzene standard to 100 mL with methanol to equal 1, 3, 5, and 10 ug/mL methanolic spiking solutions.

8.2 Measure out 25-mL portions of water samples into a series of 4-oz bottles.

8.3 Use one of the samples as a control, and fortify the remaining samples by adding appropriate aliquots of methanolic standard solutions of DNBP. Prepare each sample in duplicate.

8.4 Treat all the samples as follows:

8.4.1 Add 0.5 mL concentrated phosphoric acid and 8 g of sodium chloride.

8.4.2 Partition the sample two times for 3 minutes with 20 mL diethyl ether each time. Centrifuge and combine the ether phase to 40 mL total volume.

8.4.3 Prepare a 1- x 3-cm basic alumina column by placing 3 cm in a 1-cm-wide glass column and place a small plug of glass wool on top.

8.4.4 Pour 20 mL of the ether solution from Step 8.4.3 on the alumina column and allow it to run completely through. Discard the ether eluate.

8.4.5 Place a capsule vial under the column and elute with 5 mL then 10 mL of pH-8 water. A slight air pressure may be applied.

8.4.6 Acidify the eluate with 1 mL of 5% phosphoric acid solution.

8.4.7 Add 5 mL of diethyl ether. Cap the tube and shake for 3 minutes, then centrifuge.

8.4.8 Pipet the ether phase into a 50-mL beaker.

8.4.9 Add 1 mL of diazomethane.

8.4.10 After 30 minutes, evaporate the ether to near dryness with a gentle stream of air.

8.4.11 Take up the residue in 1 mL of hexane.

8.4.12 Prepare an acidic alumina column by placing a small plug of glass wool in a disposable capillary pipet and adding 1 inch of acidic alumina.

8.4.13 Add the hexane solution from Step 8.4.12 to the prepared column.

8.4.14 Discard the hexane eluate and elute the column with 5 mL of ethyl ether into a 5-mL volumetric flask.

8.4.15 Adjust the volume to 5 mL.

8.4.16 Chromatograph 4 uL of the ether solution as described in Section 12. Determine the apparent ppm DNBP in the fortified samples by means of the standard curve. Correct for blank, if any, by subtracting ppm of apparent DNBP of the control sample from that of the treated sample.

8.4.17 Calculate the percent recovery of DNBP from each of the fortified samples and average the results.

8.5 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Analyze 25-mL samples of water as described in Section 8 (Step 8.4), and record peak heights for each sample.

10.2 Determine the ppm DNBP in each sample by referring to the standard curve described in Section 7. Correct for blank, if any, by subtracting ppm of apparent DNBP of the control sample from that of the treated sample.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

12.3 Gas Chromatograph Column

12.3.1 Dissolve 0.75 g DC 200 in 80 mL of chloroform in a round-bottom flask. Add 15 grams 80-100 mesh Gas-Chrom Z, and boil off most of the chloroform on a steam bath. Dry the column packing on the rotary evaporator over a 50°C water bath. The liquid phase loading on the support is 5% DC 200 by weight.

12.3.2 Fill the column with packing and tamp on a solid surface such as the floor until no further settling occurs. When finished, the packing in the inlet arm should be 3 in below the injection septum. Insert a small glass-wool plug over the packing at the effluent end of the column, but not in the inlet side. Condition the column at 200°C overnight with an N_2 flow of about 100 mL/minute.

12.3.3 Injection Technique -- Fill the needle of the 10-uL syringe with benzene, eliminating all air bubbles with a rapid up-down stroke. Draw 4 uL of solution into the syringe, and inject onto the chromatographic column.

13. Calculations

13.1 Calculate the corrected apparent residue in the sample as follows:

$$\text{ppm DNBP} = \frac{\text{Net ppm DNBP}}{\text{Percent Recovery}} \times 100$$

The final 5 mL of ether solution represents 2.5 g of original sample. Therefore, 0.4 times the concentration of that solution, expressed as ug/mL, is equivalent to parts-per-million residue in the water sample.

mg/L (ppm) in original sample = ug/mL found x 0.4 x any additional dilution

14. Method Performance

Not Available.

15. References

15.1 Organic Synthesis, Col. Vol. IV, p. 250, John Wiley and Sons, Inc. (1963).

15.2 These directions are adapted from the procedure published by the Aldrich Chemical Company, Inc., 2369 North 29th Street, Milwaukee 10, WI.

15.3 R.L. McKellar, "Determination of Residues of DNBP (2-sec-butyl-4,6-dinitrophenol) in Alfalfa by Gas Chromatography: Supplement for Water," ACR 70.5.99, February 13, 1973, Dow Chemical Company, MI.

Table 1

Chromatographic Conditions

Column temperature	188°C
Injector block temperature	211°C
Detector bath temperature	218°C
Detector operating voltage	13 volts
Carrier gas	prepurified N_2 at 85 mL/min
Recorder	0 - 5 mv
Electrometer sensitivity	3.3×10^{-10} amp, full scale
Chart speed	20 in/hr

Column - U-shaped glass, 74-in x 3-mm, packed with 80/100 mesh Gas Chrom Z, 5% DC 200

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF DINOSEB IN WASTEWATER

METHOD 122

1. Scope and Application

1.1 This method covers the determination of dinoseb. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Atrazine	39033	1912-24-9
Dinoseb	—	88-85-7
Methyl parathion	39600	298-00-0
Toxaphene	39400	8001-35-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in total plant effluent waters.

1.3 The method detection limit (MDL) for atrazine, dinoseb, and methyl parathion is 0.1 mg/L. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A measured volume of water sample (1000 mL) is adjusted to pH 2 and extracted several times with chloroform. The combined extracts are evaporated to dryness on a rotary evaporator using a 55°-50°C water bath. The residue is dissolved in exactly 5 mL of acetone. Analysis is by gas chromatography using a flame ionization detector.

2.2 A rotary evaporator is used for sample concentration rather than the K-D apparatus specified in Ref. 15.2. The reason for the

switch is that Atrazine precipitates in the K-D receiver which prevents quantitative transfer of the sample.

3. Interferences

3.1 Glassware used in this and all other trace pesticides analyses is dedicated and undergoes rigorous cleanup and meticulous handling.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Precision liquid syringe, 10 uL

5.2.2 Standard laboratory glassware (chromic acid washed)

5.3 Rotary Evaporator, Rotavapor R or equivalent

5.4 Gas Chromatograph with FID-Tracor 550 or equivalent

5.4.1 Column: 6-ft x 1/4-in glass column packed with 1.95% OV-17 + 1.5% QF-1 on 80/100 mesh Gas Chrom Q

5.4.2 Integrator, Varian CDS-111 or equivalent

6. Reagents

6.1 Acetone, pesticide quality

6.2 Chloroform, pesticide quality

6.3 H_2SO_4, reagent grade

6.4 Atrazine, Dinoseb, Methyl Parathion standards

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh exactly 0.1 g Dinoseb, 0.1 g Methyl Parathion, and 0.3 g Atrazine into a 250-mL volumetric flask. Dilute to volume with acetone.

7.2.2 Using a precision syringe, inject exactly 1, 2, 4, and 6 uL standard solution on the GC. Record the integration counts and the volume of each injection for each compound. Graph the integration counts versus ug for each pesticide.

8. Quality Control

8.1 Quality control checks are performed by sample spiking. Results show 98% recovery. Also, each analysis is performed with a standard curve.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Adjust pH of exactly 1 liter effluent to pH = 2 using concentrated sulfuric acid, and place in 2-L separatory funnel.

10.2 Extract 3 times using 100-mL aliquots of chloroform. Combine chloroform layers in a 500-mL round-bottom flask and evaporate to dryness on a Rotavapor using a 55°-60°C water bath.

10.3 Dissolve the residue in exactly 5 mL Acetone.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

12.3 Inject 2-6 uL sample solution. Adjust sample size so that each pesticide value will fall on its standard curve. Note ug of each pesticide.

13. Calculations

13.1 $$\text{mg/L} = \frac{(\text{ug pesticide from standard curve})(5)}{\text{mL injected}}$$

14. Method Performance

14.1 The detection limit is approximately 0.1 mg/L for all three pesticides.

15. References

15.1 "Manual of Analytical Quality Control for Pesticides in Human and Environmental Media," by the U.S. Environmental Protection Agency (Publication Number EPA-600/1-76-017).

15.2 "Federal Register," Volume 38, Number 75, Part II, as the "National Pollutant Discharge Elimination System, Appendix A."

15.3 "Determination of Atrazine, Dinoseb, and Methyl Parathion in Plant Effluent," Standard Test Method, Vicksburg Chemical Company, Vicksburg, MS. Includes an addendum for Toxaphene.

Table 1

Chromatographic Conditions

Column Temperature	190°C
Injection Temperature	250°C
Detector Temperature	220°C
Carrier Gas	Nitrogen
Flow Rate	90 mL/min

Column - 6-ft x 1/4-in glass packed with 1.95% OV-17 + 1.5% QF-1 on 80/100 mesh Gas Chrom Q.

EPA

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Water and Waste Management

TEST METHOD

DETERMINATION OF ETHION[TM] IN WASTEWATER

METHOD 123

1. Scope and Application

1.1 This method covers the determination of ethion.

Parameter	Storet No.	CAS No.
Ethion	39398	563-12-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 A measured volume of water sample (50 mL) is extracted with hexane. The combined extract is dried over sodium sulfate, and filtered into an Erlenmeyer flask. The extract is concentrated to less than 1 mL and analyzed by gas chromatography with flame photometric detector in the phosphorus mode.

3. Interferences

3.1 The use of the detector specific for phosphorus greatly reduces the problems of interference. The detector does not respond to general organic background.

3.2 The flame photometric detector can be adjusted to respond to sulfur by a simple change of optical filters. Since Ethion[TM] contains sulfur, the question arises of how sulfur detection would work for traces of Ethion[TM]. It has been found that small amounts of sulfur compounds are present to a much greater extent than volatile phosphorus compounds in the general environment. Interference problems are therefore much greater with sulfur detection.

4. Safety

4.1 Ethion[TM] is a toxic substance that should be handled with care. It is a cholinesterase inhibitor that can be absorbed through the skin. The main hazard is contact. The vapor pressure is negligible at room temperature. The odor associated with technical Ethion[TM] is not the compound itself, but traces of organic sulfur compounds.

4.2 Protective gloves should be worn when handling Ethion[TM] or Ethion[TM] solutions. Do not allow spills to remain on bench tops; clean them up immediately. Rinse out glassware that has contained Ethion[TM] with organic solvents.

4.3 Exposure to ethion[TM] can be detected by regular measurement of blood cholinesterase levels.

4.4 All persons who become contaminated by organo-phosphates are instructed to wash thoroughly, or take a shower for fifteen minutes (if more than hands have come in contact with the chemicals), and change clothing. If there are no symptoms following the "spill," the test for cholinesterase is performed approximately 24 hours later.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Usual laboratory glassware

5.2.2 Small sample vials

5.2.3 Tuberculin syringe, 1 mL, graduated in 1/100 mL, BD No. 2004

5.2.4 Cannulas, 23 gauge, 2-in long, BD No. 1788

5.2.5 Microsyringe, Hamilton 701 N

5.2.6 Separatory funnels, 125 mL

5.2.7 Filter funnel

5.2.8 Centrifuge tubes

5.3 Filter paper

5.4 Reducing valve and needle valve controls for nitrogen system

5.5 Fume hood

5.6 Steam bath

5.7 Gas chromatograph, equipped with flame photometric detector in the phosphorus mode

5.7.1 A Hewlett-Packard Model 5830A with reporting integrator was used for this work so that operating instructions are given for that type of equipment. Any equivalent GC can be used.

5.7.2 GC column. A Teflon column, 1/4-in O.D. by 4-ft long packed with 10% SE30 on Chromasorb W, 80/100 mesh.

6. Reagents

6.1 Ethion[TM] standard of known purity

6.2 Hexane, distilled in glass

6.3 Sodium sulfate, ACS, anhydrous. Destroy traces of adsorbed organic contamination by heating in a furnace at about 500°-550°C overnight. A platinum or porcelain dish should be used to hold the salt while in the oven. Store treated reagent in air tight containers. Several small containers are preferable to a single large one.

6.4 Methanol, distilled in glass

6.5 Distilled water. Pass through activated carbon bed to remove traces of organic contamination.

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh approximately 0.1 g of Ethion[TM] into a 100-mL volumetric flask. Record the exact weight to 0.1 mg. Add hexane to the mark, and mix flask contents well.

7.2.2 Pipet 1 mL of the concentrate, prepared in Step 7.2.1 into a 100-mL volumetric flask. Dilute to the mark with hexane, and mix well.

7.2.3 Pipet 10 mL of the solution, prepared in Step 7.2.2, into a 100-mL volumetric flask. Dilute to the mark with hexane, and mix well. This will be a concentration of about 1000 ug/L. The exact concentration in micrograms per liter will be numerically equal to the exact weight (in grams) from Step 7.2.1, multiplied by 10,000.

7.2.4 Calibrate the GC by injecting 10 uL of the hexane standard from Step 7.2.3. Run according to the chromatographic conditions given in Table 1.

For Model 5830A, run the sample by "Area %," enter the external standard (ESTD) mode, 5% retention time window, the retention time and the amount (AMT). The amount will be the concentration of the standard expressed in ug/L. Rerun the standard as a sample to check the calibration.

For other instruments, determine the concentration/peak area ratio. If the same size injection (in this case, 10 uL) is used, consistently the peak area for Ethion[TM] will be proportional to the concentration.

8. Quality Control

8.1 All of the usual precautions and standard practices required for trace analyses must be followed.

8.2 Blank analyses, which include all reagents and operations except an actual sample, must be run at frequent intervals. It is difficult to control trace contamination in a laboratory where Ethion [TM] is handled. It is possible, however, to obtain a flat GC baseline.

8.3 Known water samples must be run at frequent intervals. The following procedure is provided for this purpose.

8.3.1 Weigh approximately 0.1 g of Ethion[TM] into a 100-mL volumetric flask. Record the exact weight to 0.1 mg. Add methanol to the mark, and mix the flask contents well. This will be a concentration of about 1000 ug/mL . The exact concentration in ug/mL will be equal to the exact weight (in grams) multiplied by 10,000.

8.3.2 Pipet 1 mL of the 1000-ug/mL concentrate into a 1-liter volumetric flask. Dilute to the mark with water. This will be a concentration of 1/1000 of the exact concentration from Step 8.3.1 or approximately 1 ug/mL.

8.3.3 Pipet 10 mL of the 1-ug/mL solution into a 1-liter volumetric flask. Dilute to the mark with water. This will be a concentration of 1/100 of the exact concentration from Step 8.3.2 or approximately 10 ug/L.

8.4 A fixed routine must be followed for the GC procedure to assure dependable results.

8.4.1 Inject two 10-uL samples of hexane. A flat baseline must be obtained to be certain that the syringe is clean.

8.4.2 Inject 10 uL of the standard in hexane. If the chromatogram appears normal, proceed with the calibration.

8.4.3 Rinse the syringe well with hexane.

8.4.4 Inject 10 uL of hexane.

8.4.5 Inject 10 uL of sample.

8.4.6 Rinse the syringe well with hexane.

8.4.7 Inject 10 uL of hexane.

8.4.8 Inject 10 uL of the next sample.

8.4.9 Continue with this sequence until all samples have been run.

8.4.10 After all samples have been run, inject 10 uL of the standard in hexane.

8.5 Glassware used for this operation must be kept segregated. It should be cleaned by soaking in alcoholic caustic, rinsing with water, and oven drying.

Caution: Alcoholic caustic is irritating to skin and eyes. Wear goggles when using the bath. Use tongs to place glassware in the bath, or to remove it.

8.6 Keep the bench top and general area well scrubbed. Do not use any phosphorus-containing detergents.

8.7 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow the EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Extract 50 mL of wastewater sample with 50 mL of hexane. Drain the aqueous layer into a second separator funnel. Drain the extract into a 250-mL Erlenmeyer flask.

10.2 Extract the aqueous layer with a second 50-mL portion of hexane. Drain the aqueous layer into the first funnel. Combine the extract with the previous extract in the Erlenmeyer flask.

10.3 Extract the aqueous layer with a third 50-mL portion of hexane. Discard the aqueous layer. Combine the extract with the previous extracts in the Erlenmeyer flask.

10.4 Add 5 g to 10 g of sodium sulfate to the combined extracts. Swirl the flask gently. Filter through paper into a clean dry 250-mL Erlenmeyer flask. Rinse the solid and filter with 10 mL of hexane. Add the rinsings to the combined extracts.

10.5 Place the flask with the dried extract on a steam bath in the hood. Direct a stream of nitrogen into the flask to help carry away the hexane vapors. Concentrate to a volume of about 5 mL.

10.6 Transfer the concentrate to a centrifuge tube. Use hexane to rinse the flask, adding the rinsings to the tube.

10.7 Heat the tube on the steam bath, and use nitrogen to speed up the evaporation process. Concentrate to a little less than 1 mL.

10.8 Measure the volume of concentrate. Fit a cannula on a 1-mL syringe. Draw the concentrate into the syringe. Draw a small amount of air into the syringe behind the liquid. Read the volume of the column of liquid from the graduations on the barrel of the syringe.

10.9 Transfer the concentrate to a small vial. Cap tightly, label well, and hold for GC analysis.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the GC.

12.2 Calibrate the system as described in Section 7.

12.3 For Model 5803A, enter the "XF" factor for the individual sample. See Step 13.1, "Calculations."

12.4 After the instrument has been calibrated for ESTD, or the concentration/peak area ratio has been obtained, inject 10 uL of concentrate as prepared in Step 10.9.

13. Calculations

13.1 For Model 5830A

13.1.1 Calculate the "XF" factor. When the integrator is supplied with this factor, it will automatically correct the concentration results for the concentration change effected by the hexane extraction and evaporation. Enter the factor in the integrator individually for each sample.

$$XF = \frac{\text{Vol Conc}}{50}$$

Where: Vol. Conc = Volume in mL, of concentrate from Step 10.8.

50 = Volume, in mL, of original water sample.

13.1.2 The integrator will report the concentration of Ethion[TM] in the wastewater in ug/L. Although it is not precisely correct to do so, these results can be referred to as "PPB" for most practical purposes.

13.2 For Other Instruments

13.2.1 Calculate the Ethion[TM] concentration in the injected concentrate.

$$\text{Concon} = \frac{(\text{Constd}) \times (\text{Samparea})}{\text{Stdarea}}$$

Where:

Concon = Concentration of the concentrate, in ug/L
Constd = Concentration of the standard, from Step 7.2.3
Samparea = Area of sample peak
Stdarea = Area of standard peak

13.2.2 Correct results for the concentration change during extraction and evaporation.

$$\text{PPB} = \frac{(\text{Concon}) \times (\text{Vol Conc})}{50}$$

Where:

PPB = Concentration, in ug/L, of Ethion in wastewater sample
Concon = from Step 13.2.1
Vol Conc = Volume, in mL, of concentrate from Step 10.8
50 = Volume, in mL, of original water sample

14. Method Performance

14.1 The detection limit for Ethion[TM] in water is approximately 0.5 ug/L with the use of this method of analysis.

15. References

15.1 "Ethion(R) in Wastewater," Test Method 30.3, FMC Corporation, Agricultural Chemical Division, Baltimore, MD.

Table 1

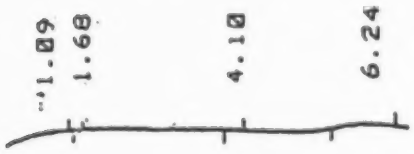
Chromatographic Conditions

Column Temperature	235°C
Injector Temperature	225°C
Auxiliary Temperature	225°C
Oven Max. Temperature	250°C
Chart Speed	1.00 cm/min
Attn 2	8
Aux sgnl	B
Slp sens	1.00
Area rej.	100
Flow A	20 (not in use)
Flow B	67
Optn.	0

Column: Teflon, 1/4-in x 4-ft, packed with 10% SE30 on Chromasorb W, 80/100 mesh.

FIGURE 1

Hexane Blank



Retention Time (min)

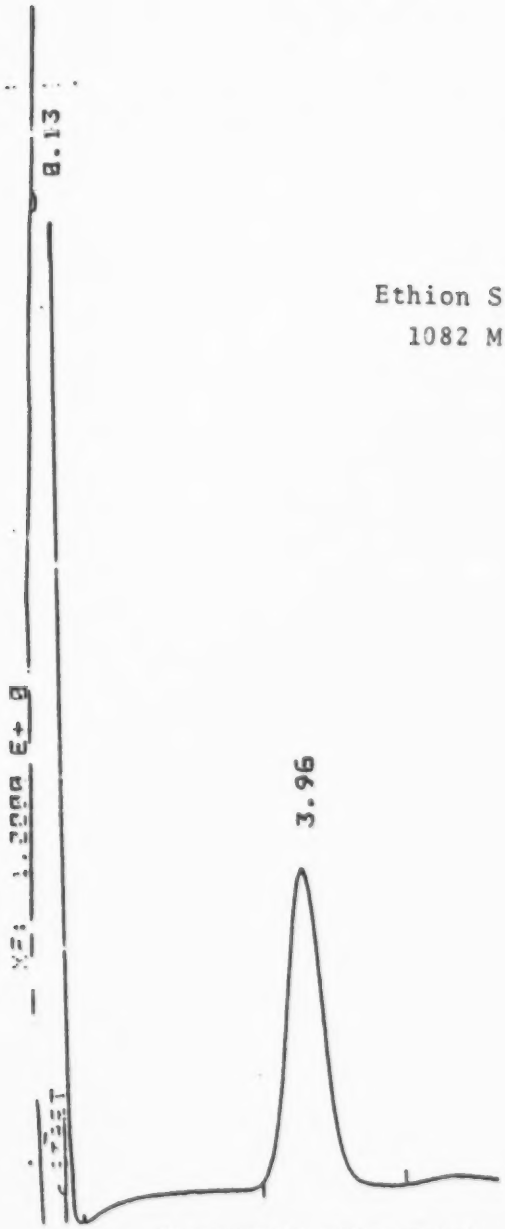


FIGURE 2
Ethion Standard in Hexane
1082 Micrograms/liter

RETENTION TIME (min)

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF ETRIDIAZOLE [TERRAZOLE(TM)] IN WASTEWATER

METHOD 124

1. Scope and Application

1.1 This method covers the determination of certain organochlorine pesticides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Etridiazole [Terrazole(TM)]	—	2593-15-9
PCNB [Terrachlor(TM)]	39029	82-68-8

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above, manufacturing impurities, and related metabolites in water.

2. Summary of Method

2.1 A measured volume of water sample (100 mL) is extracted with hexane. The extract is dried over sodium sulfate and analyzed by gas chromatography (GC) using an electron capture detector.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnel with Teflon stopcocks, 250 mL.

5.3 Perkin Elmer 3920 B gas chromatograph equipped with Ni-63 electron capture detector or equivalent.

5.3.1 Column: 6 ft of glass tubing (1/4-in O.D., 2-mm I.D.) packed with 15% SP2100 on Supelcoport 80-100 mesh. Column conditioned at 275°C with N_2 flow of 25 mL/min for about 2 hours in column conditioner.

6. Reagents

6.1 n-Hexane: Distill reagent-grade hexane (high purity) over sodium dispersed in mineral oil. Use approximately 1 mL of 50% dispersed sodium in mineral oil per liter of hexane.

6.2 Anhydrous Na_2SO_4 (granular).

6.3 Etridiazole [TERRAZOLE(TM)], Technical Grade, Olin Corporation, minimum 95% assay.

6.4 Pentachlorobenzene (PCB): Olin Corporation, obtained from PCNB process and recrystallized. Structure confirmed by infrared spectroscopy.

6.5 Hexachlorobenzene (HCB) obtained in similar manner as pentachlorobenze.

6.6 PCNB: Olin Technical Grade, 98% PCNB.

6.7 Pentachloroaniline (PCA): prepared from PCNB by reduction with zinc in ethanol - HCl, and recrystallized from ethanol.

6.8 Methyl pentachlorophenyl sulfide: prepared from PCNB by reacting with Na_2S followed by CH_3I with subsequent recrystallization from ethanol; MP 95-96°C. Structure confirmed by mass spectroscopy.

6.9 3-Dichloromethyl-5-Ethoxy-1,2,4-Thiadiazole; prepared from TERRAZOLE[TM] with $Na_2S_2O_4$. Distilled under reduced pressure, 47°C, and 0.2 mm Hg. Structure confirmed by mass spectroscopy.

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Suitable concentrations are PCB at 0.0001 ug/mL; HCB and PCNB at 0.0002 ug/mL; PCA, methyl pentachlorophenyl sulfide, TERRAZOLE[TM] and 3-Dichloromethyl-5-Ethoxyl-1,2,4-Thiadiazole at 0.0004 ug/mL. These concentrations are in the linear range of peak heights versus uL injected when 3- to 8-uL injection volumes are employed. Solutions of methyl pentachlorophenyl sulfide decompose with time; therefore, when the peak height begins to drop 15 percent, then a new diluted standard should be prepared.

8. Quality Control

8.1 Follow Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 The water sample is shaken well and a 100-mL aliquot is added to a 250-mL separatory funnel.

10.2 Extract the water with 25 mL of hexane.

10.3 The hexane layer is dried over sodium sulfate.

10.4 Analyze the hexane extract by gas chromatography.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

12.3 Inject 3-8 uL of the sample hexane extract and compare versus standards run before each sample injection.

13. Calculations

13.1 Calculate the concentration of the unknown per the following equation.

$$Cu = \frac{VS \times CS}{RS} \times \frac{Ru}{Vu} \times Vt + W$$

where Cu = Apparent ppm component in sample
VS = Microliters of standard injected

CS = Micrograms of component per mL in standard
RS = Peak height in millimeters for VS
Ru = Peak height in millimeters for unknown
Vu = Microliters of unknown injected
Vt = Volume of extracted solution
W = Volume of sample extracted

Corrected ppm component in treated sample:

$$CR = Cu \times \frac{100}{R.S.}$$

where CR = corrected ppm component in sample
Cu = ppm component in sample
R.S. = % recovery of component added to blank sample.

14. Method Performance

Not Available.

15. References

15.1 "Determination of Terrachlor(R) and Terrazole(R) in Water Samples," Standard Analytical Method, Olin Corporation.

Table 1

Chromatographic Conditions

Interface	200°C
Injection Port	200°C
Detector	225°C
Column	180°C
Carrier Gas/Flow	95% Argon/5% Methane, 45 mL/min
Attenuator	x 4
Standing Current	2.0
Chart Speed	1 cm/min

Column - 1/4-in x 6-ft glass, packed with 15% SP2100 on Supelcoport 80-100 mesh.

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TEST METHOD

DETERMINATION OF FENSULFOTHION [DASANIT(TM)] IN WASTEWATER

METHOD 125

1. Scope and Application

1.1 This method covers the determination of fensulfothion.

Parameter	Storet No.	CAS No..
Fensulfothion	--	115-90-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 A measured volume of water sample (250 mL) is adjusted to pH 6.5-7.0 and extracted with chloroform. The extract is partitioned with distilled water, dried, and evaporated to dryness. The residue is brought up in 5 mL acetone and analyzed by gas chromatography with a phosphorus-specific alkali flame detector.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Fenthion sulfoxide interferes and, if present, will appear as fensulfothion.

3.2 All glassware used for analyzing wastewater samples must be prerinsed with chloroform and not previously used for any analysis other than wastewater. DO NOT USE PLASTIC WASH BOTTLES FOR ANY SOLVENTS.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Microsyringe, 10 uL

5.3 Rotoevaporator, Buchi or equivalent

5.4 Water bath, 50°-55°C

5.5 Gas chromatograph, Varian Model 1400 or equivalent, equipped with a phosphorus detector, a 3-ft x 1/8-in O.D. glass column packed with 3% OV-225 on Gas Chrom Q 80/100 mesh, and glass injector and detector inserts. (If the ends of the column are long enough to be used as inserts, the glass inserts are not necessary.) Note: Use only Teflon-backed septa, Supelco No. 2-0459.

6. Reagents

6.1 Acetone, nanograde

6.2 Chloroform, nanograde

6.3 Hydrochloric acid, 1N -- Carefully add 21 mL of concentrated hydrochloric acid into a 250-mL glass-stoppered graduated cylinder containing approximately 200 mL of distilled water. Dilute to 250 mL with distilled water and mix thoroughly.

6.4 Mineral oil solution, 2.5% -- Dilute 25 mL of mineral oil, Fisher #0-120 or equivalent, to 1000 mL with nanograde chloroform and mix thoroughly.

6.5 Sodium hydroxide, 1N

6.6 Sodium sulfate, anhydrous, ACS

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Fensulfothion (DASANIT), 0.10% -- Weigh 0.110 - 0.115 g of Fensulfothion (DASANIT) (88% minimum purity) into a 100-mL volumetric flask. Dilute to volume with acetone, stopper,

and mix thoroughly. This solution may be used for one week only.

7.2.2 Pipet a 1-mL aliquot of the 0.10% Fensulfothion (DASANIT)solution into a 100-mL volumetric flask, dilute to volume with acetone, and mix thoroughly. Label this solution "10 ng." Prepare fresh daily.

7.2.3 Pipet 5-, 10- and 20-mL aliquots of 10-ng standard of Step 7.2.2 into separate 100-mL volumetric flasks. Dilute to volume with acetone and mix. Label these solutions 500, 1000, and 2000 pg, respectively. Prepare fresh daily.

7.2.4 Continue analysis according to TM A-54.12 (Mobay reference method, not enclosed herein).

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample by shaking, then proceed immediately to Step 10.2.

10.2 Measure 250 mL of the well-mixed sample into a 500-mL separatory funnel.

10.3 If the pH is above 7.0 or below 6.0, adjust it to pH 6.5-7.0 with 1N hydrochloric acid or 1N sodium hydroxide using pH indicator paper or a pH meter.

10.4 Extract three times by vigorously shaking for 1 minute each time with fresh 50-mL portions of nanograde chloroform. Collect the three chloroform extracts in another 250-mL separatory funnel. If an emulsion forms, centrifuge the emulsified layer and add the clear chloroform layer, obtained by centrifuging, to the second separatory funnel. Return the water layer to the first separatory funnel.

10.5 Add 50 mL of distilled water to the combined chloroform extracts in the second separatory funnel and shake for one minute.

10.6 Filter the chloroform layer through a funnel containing a glass-wool plug and 4 to 5 g of anhydrous sodium sulfate (pre-rinsed with 10-mL of chloroform), into a 300-mL 24/40 boiling flask. Rinse the sodium sulfate three times with 10 mL of chloroform. Add 10 mL of the mineral oil solution to the boiling flask.

10.7 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.8 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.9 Pipet 5 mL of acetone into the flask from Step 10.8, stopper immediately, and rotate the flask so that the acetone washes down the inside of the flask. DO NOT allow the acetone to get on the neck or stopper.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Continue the analysis according to TM A-54.12 (Mobay reference method, not enclosed herein).

13. Calculations

Not Available.

14. Method Performance

Not Available.

15. References

15.1 "Determination of Dasanit(R) in Waste Water by GLC," Analytical Method TM 13-34.38, Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO.

Table 1

Chromatographic Conditions

Attenuation	X32
Cell voltage	Flame
Detector temperature	210-230°C (do not exceed 230°C)
Electrometer range	10^{-12}
Gas flows:	
Carrier gas	Helium, 20 mL/min
Air	230 mL/min
Hydrogen	40 mL/min
Injector temperature	200°-215°C (do not exceed 215°C)
Oven temperature	230°C

Column - 3-ft x 1/8-in O.D. glass, packed with 3% OV-225 on Gas Chrom Q 80/100 mesh.

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TEST METHOD

DETERMINATION OF FENTHION IN WASTEWATER

METHOD 126

1. Scope and Application

1.1 This method covers the determination of fenthion.

Parameter	Storet No.	CAS No.
Fenthion	--	55-38-9

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 A measured volume of water sample (250 mL) is adjusted to pH 6.5-7.0 and extracted with chloroform. The extract is partitioned with distilled water, dried, and evaporated to dryness. The residue is brought up in 5 mL acetone and analyzed by gas chromatography with a phosphorus-specific alkali flame detector.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 BAY 25064 interferes and, if present, will appear as fenthion.

3.2 All glassware used for analyzing wastewater samples must be prerinsed with chloroform and not previously used for any analysis other than wastewater. DO NOT USE PLASTIC WASH BOTTLES FOR ANY SOLVENTS.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Microsyringe, 10-uL

5.2.2 Rotoevaporator, Buchi or equivalent

5.3 Water bath, 50°-55°C

5.4 Gas chromatograph, Varian Model 1400 or equivalent, equipped with a phosphorus detector, a 3-ft x 1/8-in O.D. glass column packed with 15% OV-17 on Chromosorb W, DMCS 60/80 mesh, and glass injector and detector inserts. (If the ends of the column are long enough to be used as inserts, the glass inserts are not necessary.) Note: Use only Teflon-backed septa, Supelco No. 2-045.

6. Reagents

6.1 Acetone, nanograde

6.2 Chloroform, nanograde

6.3 Hydrochloric acid, 1N -- Carefully add 21 mL of concentrated hydrochloric acid into a 250-mL glass-stoppered graduated cylinder containing approximately 200 mL of distilled water. Dilute to 250 mL with distilled water and mix thoroughly.

6.4 Mineral oil solution, 2.5% -- Dilute 25 mL of mineral oil, Fisher #0-120 or equivalent, to 1000 mL with nanograde chloroform and mix thoroughly.

6.5 Sodium hydroxide, 1N -- Carefully dissolve 10 g of sodium hydroxide pellets, ACS, in 250 mL of distilled water and mix thoroughly.

6.6 Sodium sulfate, anhydrous, ACS

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Fenthion, 0.10% -- Weigh 0.100 - 0.110 g of fenthion (95% minimum purity) into a 100-mL glass-stoppered graduated cylinder. Dilute to 100 mL with acetone, stopper, and mix thoroughly. This solution may be used for one week only.

7.2.2 Pipet a 1-mL aliquot of the 0.10% fenthion solution into a 100-mL volumetric flask, dilute to volume with acetone, and mix thoroughly. Label this solution "10 ng." Prepare fresh daily.

7.2.3 Pipet 5-, 10- and 20-mL aliquots from the 10-ng standard of Step 7.2.2 into separate 100-mL volumetric flasks. Dilute to volume with acetone and mix. Label these solutions 500, 1000, and 2000 pg, respectively. Prepare fresh daily.

7.2.4 Continue the analysis according to TM A-54.12 (Mobay reference method, not enclosed herein).

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample by shaking, then proceed immediately to Step 10.2.

10.2 Measure 250 mL of well-mixed sample into a 500-mL separatory funnel.

10.3 If the pH is above 7.0 or below 6.0 adjust it to 6.5 - 7.0 with 1N hydrochloric acid or 1N sodium hydroxide using pH indicator paper or a pH meter.

10.4 Extract three times by vigorously shaking for 1 minute each time with fresh 50-mL portions of nanograde chloroform. Collect the three chloroform extracts in another 250-mL separatory funnel. If an emulsion forms, centrifuge the emulsified layer and add the clear chloroform layer, obtained by centrifuging, to the second separatory funnel. Return the water layer to the first separatory funnel.

10.5 Add 50 mL of distilled water to the combined chloroform extracts in the second separatory funnel and shake for one minutte.

10.6 Drain the chloroform layer through a funnel containing a glass-wool plug and 4 to 5 g of anhydrous sodium sulfate (pre-rinsed with 10 mL of chloroform), into a 300-mL 24/40 boiling flask. Rinse the sodium sulfate three times with a 10-mL portion of

chloroform, and add 10 mL of 2.5% mineral oil solution into the flask.

10.7 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.8 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.9 Pipet 5 mL of acetone into the flask from Step 10.8, stopper immediately, and rotate the flask so that the acetone washes down the inside of the flask. DO NOT allow the acetone to get on the neck or stopper.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Continue the analysis according to TM A-54.12 (Mobay reference method, not enclosed herein).

13. Calculations

Not Available.

14. Method Performance

Not Available.

15. References

15.1 "Determination of Fenthion in Waste Water by GLC," Analytical Method TM B-34.37, Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO.

Table 1

Chromatographic Conditions

Attenuation	X32
Cell voltage	Flame
Detector temperature	230°-240°C (do not exceed 250°C)
Electrometer range	10^{12}
Gas flows:	
Carrier gas	He, 20 mL/Min
Air	230 mL/min
Hydrogen	40 mL/min
Injector temperature	230°-240°C (do not exceed 250°C)
Oven temperature	235°C

Column: 3-ft x 1/8-in glass, packed with 15% OV-17 on Chromosorb W, DMCS 60/80 mesh.

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TEST METHOD

DETERMINATION OF GLYPHOSATE IN WASTEWATER

METHOD 127

1. Scope and Application

1.1 This method covers the determination of glyphosate.

Parameter	Storet No.	CAS No.
Glyphosate	39941	1071-83-6

1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of glyphosate in industrial effluent.

1.3 The method detection limit (MDL) is 50 ug/L (ppb) of glyphosate standard with 5% of full-scale deflection. The MDL for a specific wastewater may be different depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A known volume of glyphosate industrial effluent, including noncontact cooling waters, is applied to AG50W-X8 (Bio-Rad) cation exchange column and eluted with two bed volumes of water. The collected effluent is preconcentrated and/or injected into a HPLC using a Technicon-based ninhydrin/post-column detector. The peak height of the resulting glyphosate is determined and compared with peak heights obtained from standards.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Ammonia, as well as primary and secondary amines, will have a significant response with ninhydrin reagent, and, therefore,

prevent detection of the presence of the glyphosate peak in the chromatogram.

3.2 A sample clean-up using packed AG50W-X8 strong cation exchange column (Bio-Rad Inc.) will eliminate the interferences of amines, organic amines, and amino acid derivatives. A preconcentration factor of 20 (10 mL of waste effluent concentrated to 0.5 mL) for dilute industrial effluents associated with glyphosate production is unequivocally free of interferences.

3.3 Equipment used in this analysis (columns, filters, glassware, etc.) should be limited to trace analysis only. This will eliminate any possibility of residual contamination at these low levels.

3.4 The employment of 0.04-0.06% NaOCl for washing the glassware is to remove any remaining trace levels of glyphosate. The glassware should be rinsed with copious amounts of distilled water before application. Routine change of vacuum pump oil is necessary if contamination has been observed.

4. Safety

4.1 Follow EPA Safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Pipets, 10-100 uL, 100-1,000 uL, and 1-5 mL delivery pipets with disposable tips.

5.2.2 Volumetric flasks, 100 mL or convenient sizes for making standards or dilution.

5.3 Buffer pump, Waters 6000A pump, flow rate 0.6 mL/min.

5.4 Injector, Waters Intelligent Sample Processor (WISP).

5.5 Ninhydrin Reagent Technicon proportionation pump.

5.6 Reactor, Technicon research cartridge or oil bath controlled at 95°C.

5.7 Detector, Technicon Single Channel Colorimeter with 570-nm optical interference filter and 1.5-mm x 55-mm flow cell.

5.8 Electronic Filter -- Spectrum 1021 filter and amplifier.

5.9 Recorder, Fisher, 100 mv-range.

5.10 HPLC Column, DuPont Zorbax SAX, 15-cm x 4.6-mm (853952703).

5.11 Vacuum Evaporation Setup, including pump, condensor, rotator, heating plant, and receiving flask.

5.12 CDC 1700 Computer System.

5.13 AG50W-X8, 100-200 mesh, Econo Column (Bio-Rad, 731-6213).

6. Reagents

6.1 Dimethysulfoxide (Pierce, 20687)

6.2 Ninhydrin (Pierce, 21001)

6.3 Lithium Acetate pHix[TM] Buffer, 4M $Li^{\oplus}$; pH 5.20 (Pierce, 27203)

6.4 Hydrindantin Dihydrate (Pierce, 24000)

6.5 Methanol (Burdick and Jackson)

6.6 H_3PO_4 (85%, Fisher A-242)

6.7 KH_2PO_4 (Mallinckrodt, 7100)

6.8 Brij[TM] 35, 30% aqueous solution (Fisher CS-285-2)

6.9 Argon

6.10 NaOCl (4-6%, Fisher SOS-290)

6.11 H_2SO_4 (Fisher, A-300)

6.12 Preparation of Reagent

6.12.1 HPLC Buffer Solution -- Dissolve 10 g KH_2PO_4 in 3.8 L of 160 mL methanol/deionized water; adjust the solution to pH 2.3 with 85% H_3PO_4. Normal HPLC degassing/filtration procedures are followed.

6.12.2 Ninhydrin Solution -- Combine 1,100 mL of dimethylsulfoxide (DMSO), 800 mL of deionized water, 400 mL of 4.0M (pH 5.2) lithium acetate, and bubble argon through the solution for approximately 15 minutes. Add 32 g of ninhydrin to the solution with stirring. In a separate beaker dissolve 1.6 g of hydrindantin solution in two portions of 50 mL DMSO with a few mL of previously prepared ninhydrin solution, and add together with argon bubbling. An additional 15 minutes for argon deoxygenation is required before application.

6.12.3 Wetting Reagent for Autoanalyzer -- Dissolve 10 mL Brij in 800 mL of deionized water; a few drops of concentration H_2SO_4 are added to make acidic solution.

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 A stock solution of 1,000 ug/mL (ppm) is prepared by dissolving 100 mg of 3X recrystallized analytical grade of glyphosate in 100 mL deionized water and stored in the refrigerator.

7.2.2 A series of working standards (0.2, 0.4, 0.6, 0.8, and 1.0 ug/mL) is prepared weekly by appropriate dilutions.

7.2.3 A series of external standards (0.2-1.0 ug/mL) is prepared and analyzed under the same HPLC conditions and on the same day as unknown samples. A blank industrial effluent sample and water also have to be included in the sample pretreatment and HPLC analysis to ensure the absence of glyphosate contamination via sample manipulation. It is recommended that only the lowest (0.2 ug/mL) concentration of glyphosate standard be employed for the determination of glyphosate less than 30 ug/L in the waste sample. This effectively eliminates any contamination due to syringe and needle carryover of standards.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 The AG50W-X8 resin column is washed with 15 mL of deionized water before application.

10.2 For glyphosate concentrations greater than 300 ug/L, 1.0 mL of sample is applied to the column and then eluted with 5 mL of deionized water. The collected eluent (6 mL) is ready for HPLC injection after complete mixing.

10.3 For glyphosate concentrations less than 300 ug/L, 10.0 mL of sample is applied to the column and then eluted with 5 mL of deionized water. The collected eluent is evaporated to dryness

under vacuum at 50°-60°C. Deionized water (0.5 mL) is added to the residual, which is then ready for HPLC injection.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Construction of Ninhydrin Reactor -- Refer to Figure 1.

12.4 HPLC Injection -- An alternating injection between sample and standard is carried out throughout the analysis.

12.5 A typical chromatogram of glyphosate standard, blank, and waste effluent sample is included in Figure 2. It has been observed that the retention time of glyphosate in waste effluent samples is approximately 60 seconds less than the immediately preceding glyphosate standard.

13. Calculations

13.1 For glyphosate concentrations greater than 0.4 mg/L (ppm), the actual concentration of glyphosate in waste sample mg/L (ppm) =

(ppm glyphosate from calibration curve) x 6 (dilution factor)

13.2 For glyphosate concentrations less than 300 ug/L (ppb), the actual concentration of glyphosate in waste sample ug/L (ppb) =

$$\frac{\text{(peak height of sample)}}{\text{(average peak height of 200-ug/L standard)}} \times 200 \text{ ug/L}$$

14. Method Performance

14.1 The method was validated over the range of 5-30 ug/L with a preconcentration factor of 20, and 0.3-10 mg/L with a dilution factor of 6. The probable linear range is 0.05 to 10 mg/L with glyphosate standards.

14.2 The detection limit is 50 ug/L of glyphosate standard with 5% of full scale (S/N=2).

14.3 The laboratory validation of this method was carried out using synthetic glyphosate industrial waste sample. The components of the mixture are listed in Table 2. This was necessary due to currently large (ppm) quantities of glyphosate being discharged and so that future effects of pretreatment technology on glyphosate industrial effluent could be taken into account.

14.4 The pooled coefficient of variation ($\overline{CV}$) for the analytical method in the range of 5-30 ug/L and 0.3-10 mg/L range of glyphosate in 002 sump is 0.0788 and 0.0366, respectively.

14.5 The average values obtained in the 5-30 ug/L and 0.3-10 mg/L range of glyphosate in the appropriate industrial effluent were 20 and 5 percent less than the "true" values, respectively. The difference between the "found" and "true" concentrations is not due to a bias in the sampling and analytical method but rather due to a random variation of the experimentally determined "true" concentration. Therefore, no recovery should be applied to the final results in Section 13.

14.6 The HPLC/ninhydrin reaction post-column detector provides the required selectivity and sensitivity for trace-level glyphosate determination.

14.7 The disadvantage of the method is the manual sample cleanup and evaporation/preconcentration step. Therefore, the precision of the method is limited by the reproducibility of each operational step.

15. References

15.1 "Method for the Determination of Glyphosate in Industrial Effluents," Monsanto Agricultural Products, Co., St. Louis, MO.

Table 1

Chromatographic Conditions

Buffer Flow Rate	0.6 mL/min
<u>WISP Operation Parameters</u>	
Injection Volume	200 uL
Run Time	24 minutes
Number of Injections	1
Detector - DAMP 2, Std. Cal. 1.00	
Electronic Filter, Cutoff Frequency	0.01.
Attenuation	2.0.
Recorder - Chart Speed	0.25 cm/min

Table 2

Composition of the Synthetic Glyphosate
Industrial Waste Sample

Component	Composition (ppm)
1. Glyphosate Intermediate Raw Material	30
2. PO_4^{3-}	30
3. NH_3	100
4. NO_3^-	100
5. SO_4^{2-}	200
6. Cl^-	200
7. Na^+	200
8. Ca^{2+}	50
9. Mg^{2+}	10
10. Monoethanolamine	10

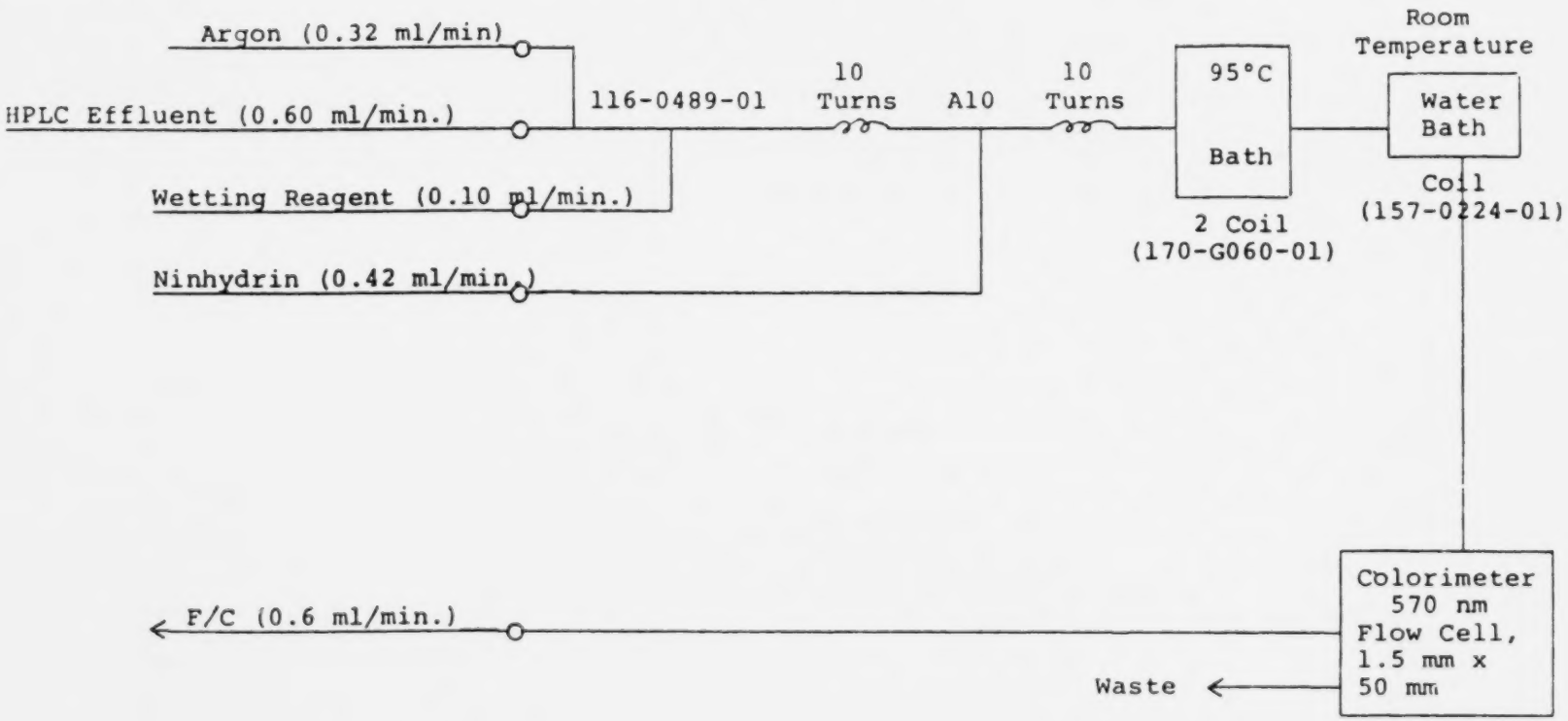


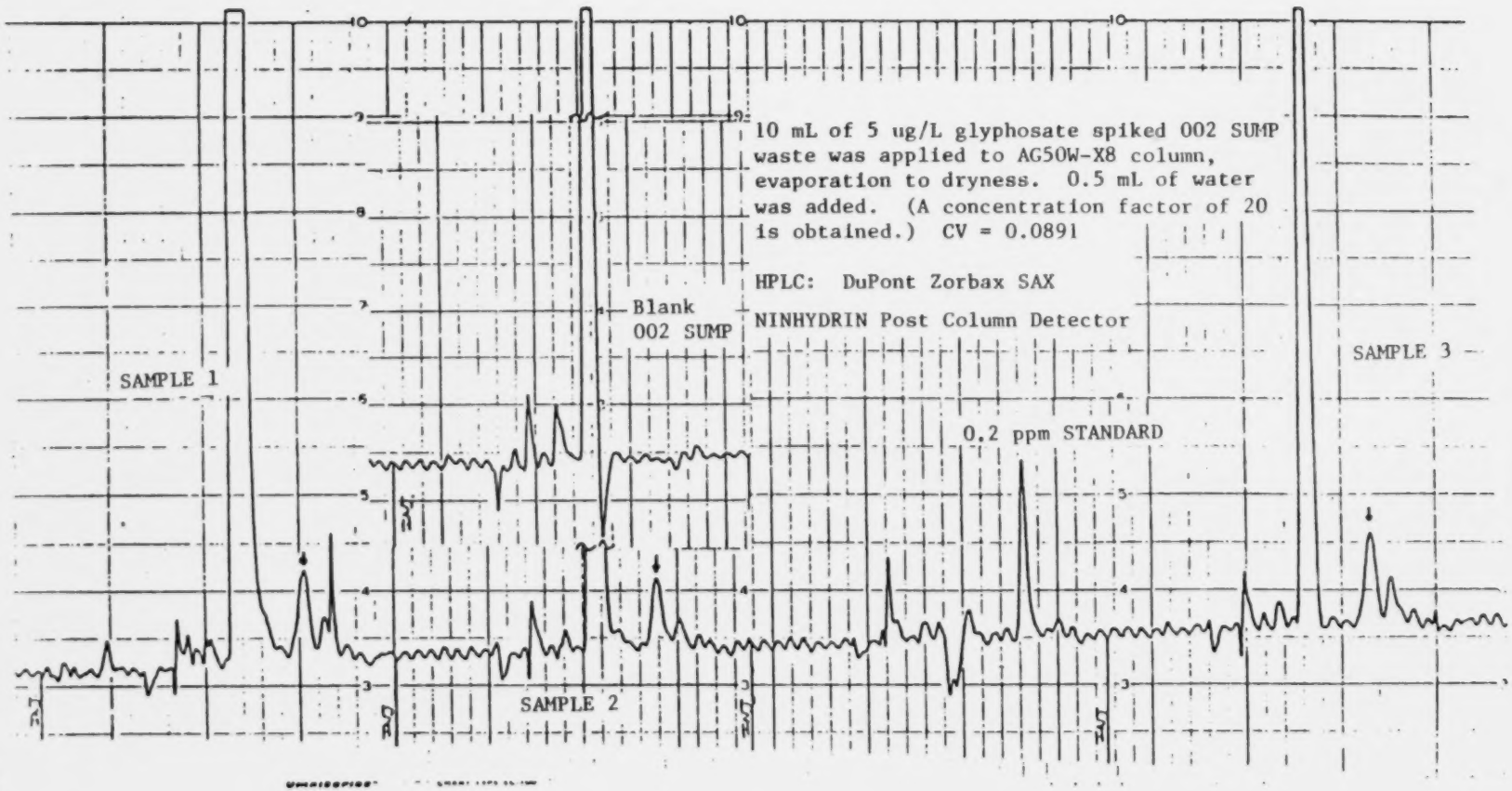
FIGURE 1.

The Hydraulic System of Technicon Autoanalyzer Based Ninhydrin Reactor

FIGURE 2.

CHROMATOGRAMS OF GLYPHOSATE STANDARDS,

INDUSTRIAL EFFLUENT AND BLANK



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TEST METHOD

DETERMINATION OF MANCOZEB [DITHANE M-45(TM)] IN WASTEWATER

METHOD 128

1. Scope and Application

1.1 This method covers the determination of mancozeb (Dithane M-45[TM]) and other carbamates.

Parameter	Storet No.	CAS No.
Mancozeb [Dithane M-45(TM)]	—	8018-01-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above and other dithiocarbamates in various samples, including wastewater streams from manufacturing operations.

1.3 The method detection limit (MDL) for mancozeb is 10 ug/L. The MDL for a specific wastewater may differ from this value depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A known weight of sample is quantitatively decomposed to carbon disulfide (CS_2) by refluxing the sample in dilute HCl in the presence of stannous chloride ($SnCl_2$). The liberated CS_2 is swept by an air stream through a series of purification traps and absorbed in ethanol at dry-ice temperature. The CS_2 is measured by gas chromatography using a flame-photometric detector in the sulfur mode. For confirmatory CS_2 analysis an aliquot of the ethanol solution is injected into a GC-MS system. The CS_2 digestion apparatus of the standard Keppel colorimetric method is used with a series of trapping tubes containing Mallcosorb[TM], lead acetate, sulfuric acid, and ethanol.

2.2 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Decomposition - distillation - absorption train (See Figure 1).

5.2.2 Gas chromatograph - equipped with a Tracor flame photometric detector with sulfur filter (394 nM) and 6-ft x 4-mm I.D. coiled glass column packed with Tenax GC 60-80 mesh.

6. Reagents

6.1 Mancozeb (Dithane M-45) Standard -- Commercial Dithane M-45 (use assay value from official formulation analysis to calculate active ingredient weight to take for preparation of spiking standard).

6.2 Na_4 EDTA Solution -- Dissolve 100 g of ethylenedinitrilo tetraacetic acid, tetrasodium salt (MCB #E x 550) in 800 mL distilled water and dilute to 1L with distilled water.

6.3 Water, distilled and deaerated -- Deaerate approximately 4 liters of distilled water by bubbling nitrogen gas through it at 50 to 100 mL per minute for 10 to 15 minutes.

6.4 95% Ethanol -- Denatured, commercial formula 3-A (Publicker Industries, Inc.).

6.5 First Absorber - Mallcosorb absorbent, 30/50 mesh, (Mallinckrodt #6077) for removal of acidic gases (CO_2, H_2S, etc.).

6.6 Second Absorber - 30% lead acetate solution for removal of remaining H_2S. Add 30 g lead acetate trihydrate (Fisher Scientific #L-33) to distilled H_2O in a 100-mL volumetric flask and dilute to volume.

6.7 Third Absorber -- Concentrated sulfuric acid (ACS reagent grade) for removal of alkaline gases and water vapor (water appearing in cryogenic capillary tubing of Absorber #4 will block air flow).

6.8 Fourth Absorber -- 95% ethanol held in bath of dry-ice/3-A ethanol.

6.9 Stannous chloride reagent -- Weigh 40 g of stannous chloride dehydrate (Fisher Scientific #T-142) and transfer it to a 100-mL volumetric flask. Dissolve and dilute to the mark with distilled water. Note: Prepare this solution within two hours of use, and discard remainder after use.

7. Calibration

7.1 Establish GC operating conditions equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Carbon disulfide standard solution for preparing GLC standard curve. Make up 95% ethanol solutions of 0.4, 0.3, 0.2, 0.15, and 0.0 ug/mL of carbon disulfide for standard curve developed using 10-uL injections.

7.2.2 Inject 10 uL of standard CS_2 solutions.

7.2.3 Read the amount of CS_2 from a calibration curve prepared by plotting peak height (chart units) versus varying nanogram (or alternatively ug/mL) levels of CS_2 standards injected.

(Establish a standard curve before each series of sample injections.)

8. Quality Control

8.1 It is advisable to run a reagent blank (standard run with all reagents but no sample) before starting a series of analyses, to demonstrate clean apparatus and guard against false positive results. A reagent blank should also be run following an abnormally high sample. From time to time the apparatus should be disassembled, cleaned, adsorbents replaced, etc., especially if reagent blanks give positive results.

8.2 Freshly prepare a stock standard of 500 ug/mL mancozeb (Dithane M-45) in 10% w/v aqueous tetrasodium ethylenediaminetetraacetate (Na_4EDTA). Make appropriate dilutions with distilled deaerated water to obtain the desired level of the spiking standard. Example: To prepare a 5-ug/mL standard, dissolve 0.05 g active ingredient (the acutal weight will be slightly more than 0.05 g depending on the percent active ingredient in 100 mL of 10% w/v aqueous Na_4 EDTA, and dilute 1 mL of this solution to 100 mL with distilled deaerated H_2O. Add the appropriate spiking aliquot to sample just prior to beginning decomposition step. [Note: EBDC

in Na EDTA solutions should not be stored due to decomposition (Ref. 16.4).]

8.3 Determine recovery by adding an aliquot of mancozeb in Na_4 EDTA spiking standard to the weighed sample in the digestion flask. Correct recovery for control when the corresponding control or unfortified sample produces a significant value.

8.4 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Sample Preparation

10.1.1 No sample preparation is necessary for wastewater except to stir or agitate so that a representative subsample is taken for analysis. The standard subsample is 50 g.

10.2 Decomposition-Distillation-Absorption

10.2.1 Transfer the sample to the 1,000-mL digestion flask using distilled and deaerated water. Add sufficient water as needed to give a volume of about 400 mL. Add boiling chips, 40 mL of concentrated HCl, and 10 mL of 40% aqueous $SnCl_2$ solution.

10.2.2 In the first absorber tube place 30 cc of Mallcosorb powder. To the second absorption tube add 10 mL of 30% aqueous lead acetate reagent, and to the third, add 5 mL of concentrated H_2SO_4. Add 5 mL of 95% ethanol to the fourth adsorber (CS_2 trap). Cool the CS_2 trapping tube by immersing in a bath of dry-ice/3-A ethanol.

10.2.3 Connect the equipment, start water flow through reflux condensor, and apply gentle vacuum to outlet of CS_2 trap to draw air through the system at a rate of about 60 mL/min. Turn on heating mantles and bring flask contents to boiling. Continue refluxing for about 45 minutes.

10.2.4 Turn off heating mantle. Disconnect the CS_2 trap from the vacuum and the dry-ice bath and let stand at room temperature for about 1 hour before diluting sample to 5 mL using 95% ethanol.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

12.3 By syringe remove a 10-uL aliquot of final ethanol sample and inject into the gas chromatograph set at the described conditions. Measure the height of the peak of CS_2 in the chromatogram and calculate the concentration from the standard curve.

13. Calculations

13.1 The amount of mancozeb (Dithane M-45) in the sample is calculated from the following equations:

1) $$\text{ng } CS_2 \text{ (from curve)} \times \frac{5 \text{ mL (EtOH trap)}}{10 \text{ uL (Inj. Vol.)}} \times 1.78 \frac{\text{MW mancozeb}}{2 \text{ MW } CS_2}$$

= Total ug mancozeb (Dithane M-45) found in sample taken for digestion

2) ug/g (ppm) mancozeb (Dithane M-45)=

$$\frac{\text{ug mancozeb (Dithane M45) found}}{\text{g of sample taken for digestion x recovery factor}}$$

14. GC/MS Confirmation

14.1 For CS_2 confirmation, inject an aliquot of the final ethanol sample into a GC/MS system having similar GC column operating parameters as those described for the flame photometric GC system. (Semi-quantitative MS estimates are made based on single ion monitoring of sample vs. standard ions at mass 75.9).

15. Method Performance

15.1 Recoveries -- Table 2 presents recovery data by the described method. Example chromatograms (Figures 2 and 3) and the corresponding calibration curve (Figure 4) are also presented.

15.2 Limit of Detection -- The minimum detectable amount is about 0.5 ng of CS_2 in 10 uL of injected solution. Based on a 5-mL final volume of ethanol sample, the detection limit is about 0.5 ug of total Mancozeb (Dithane M-45) in the sample taken for digestion. Based on these parameters the limit of detection is 0.010 ug/g for a 50-g sample. The limit of detection can be lowered, if necessary, by increasing the size of the sample. Conversely, if

high concentrations are expected it may be desirable to use a smaller sample, or to dilute the ethanol solution of trapped CS_2 before injection into the gas chromatograph.

16. References

16.1 G.E. Keppel, J.A.O.A.C. 54, 528-532 (1974).

16.2 Tokyo Organic Chemical Industries, Ltd., TOCIL Memo TR-78-9-11, T.ISO to H. Kuyama, 9/11/78, Anal. Method for Dithane M-45 Residues.

16.3 "A Gas Chromatographic Method for Measurement of Dithane M-45 or other Dithiocarbamate Fungicides in Wastewater or Natural Waters," TR36F-82-20, Rohm and Haas Comapny, Spring House, PA.

16.4 B.D. Ripley, Bull Envir. Cont. Tox. 22, 182-189 (1979).

Table 1

Chromatographic Conditions

Column temperature		140°C
Injection temperature		140°C
Detector temperature		190°C
Carrier Gas/Flow		nitrogen, 30 mL/min
	Air	80 mL/min
	Oxygen	28 mL/min
	Hydrogen	110 mL/min
Recorder Speed		5 cm/min
Range		100 mv f.s.

Column: 6-ft x 4-mm I.D. coiled glass, packed with Tenax BC 60-80 mesh

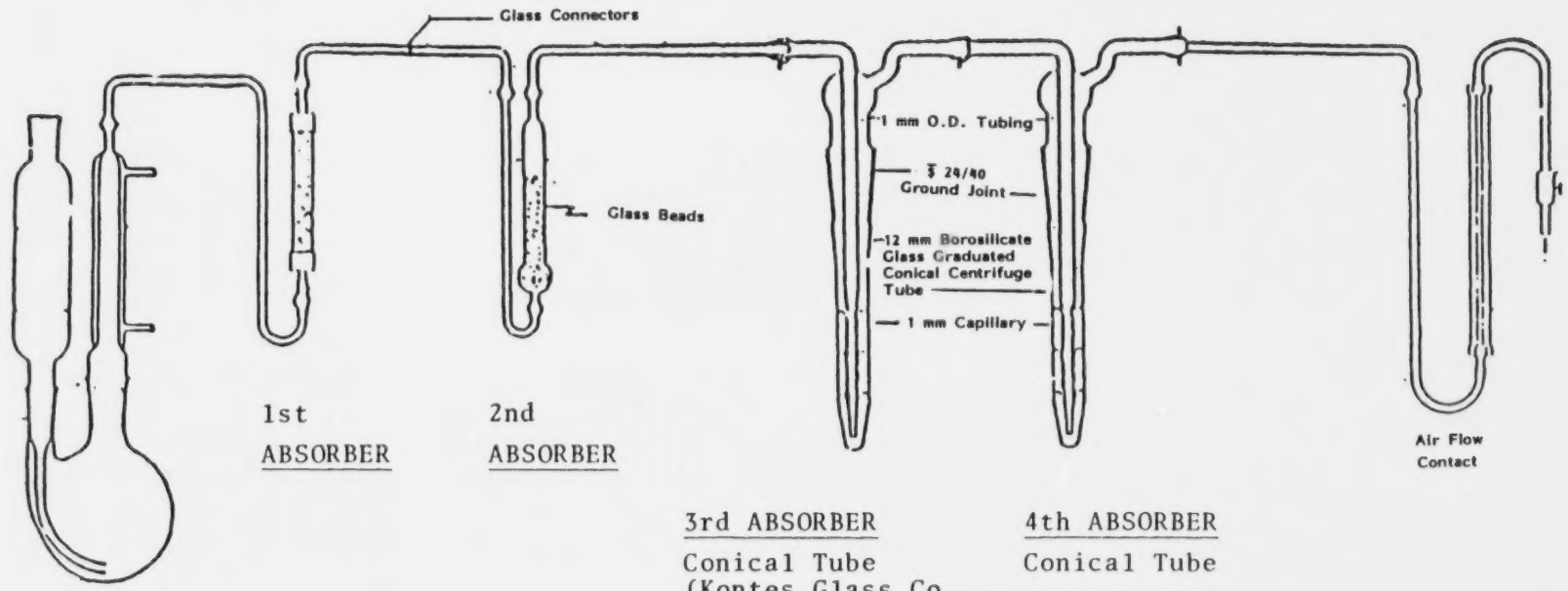
Table 2

Recovery of Fortifications of Mancozeb (Dithane M-45)

Concentration Added ug/g (ppm)	Recovery %
1.0	85.4
0.5	87.2
5.0	71.6
0.1	71.2
Average	79 ± 8

FIGURE I

DECOMPOSITION-DISTILLATION-ABSORPTION APPARATUS FOR DETERMINATION OF DITHIOCARBAMATE RESIDUES



1000 ml
Digestion Flask

1st
ABSORBER

2nd
ABSORBER

3rd ABSORBER

Conical Tube
(Kontes Glass Co.
#9935 Part E)

+

Tubing Piece
(Kontes Glass Co.
#9935 Part D)

4th ABSORBER

Conical Tube

+

Tubing Piece

(Immerse trap in dewar
containing dry-ice/3-A-ethanol)

CS_2
STANDARD
3.17 Ng/10 ul

CS_2
STANDARD
2.03 Ng/10 ul

CS_2
STANDARD
1.52 Ng/10 ul

CS_2
STANDARD
1.02 Ng/10 ul

CS_2
STANDARD
0.51 Ng/10 ul

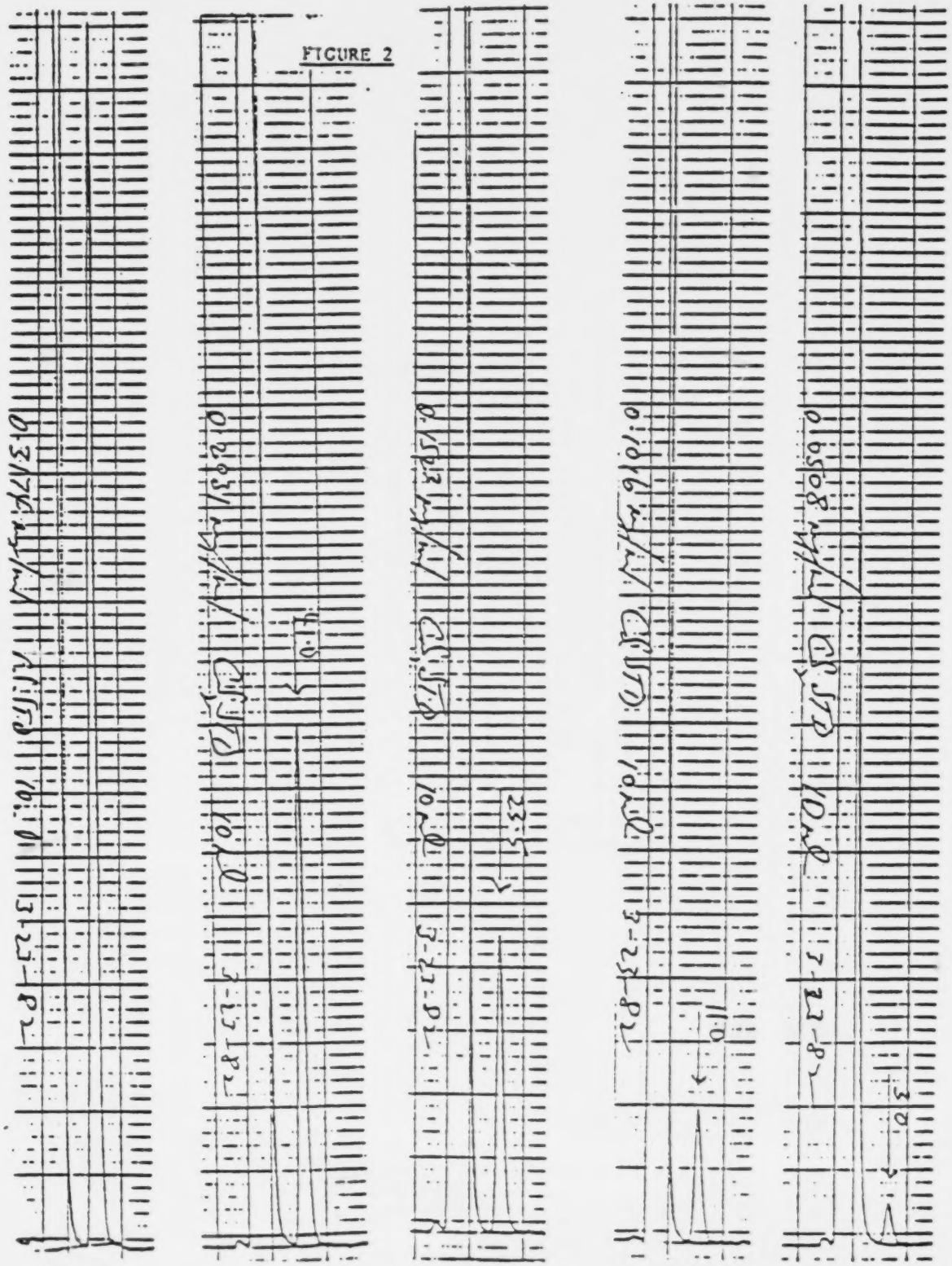


FIGURE 2

DITHANE M-45
SPIKE @ 1.0 ppm
ADDED TO
REAGENT BLANK

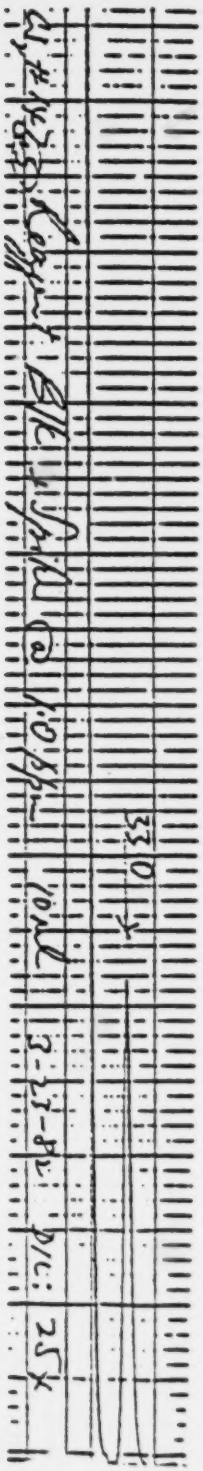
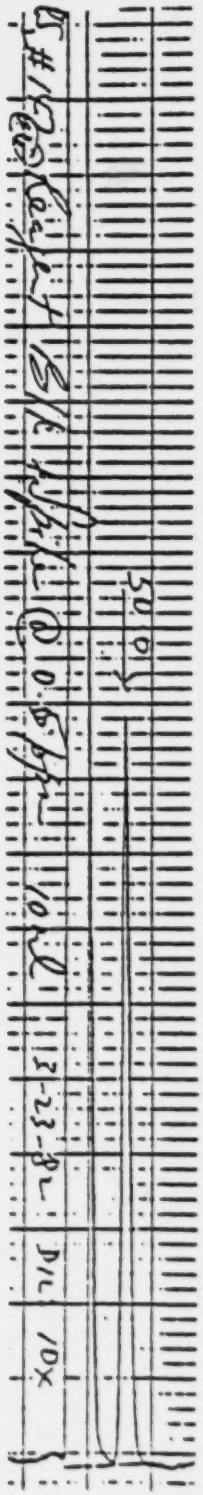


FIGURE 3

DITHANE M-45
SPIKE @ 0.5 ppm
ADDED TO
REAGENT BLANK



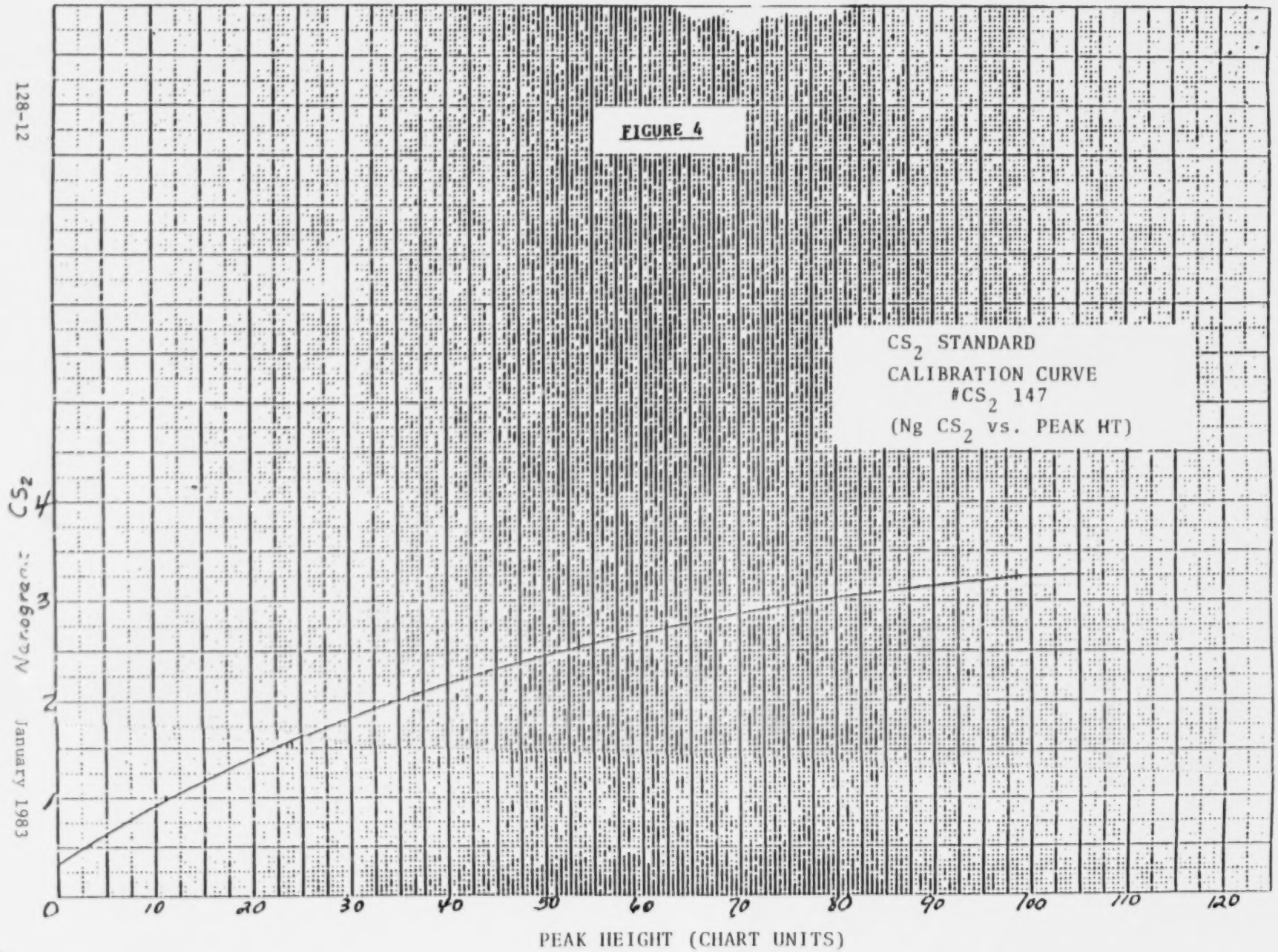


FIGURE 4
CS2 STANDARD
CALIBRATION CURVE
#CS2 147
(Ng CS2 vs. PEAK HT)
CS2
Nanogram
1
2
3
4
0
10
20
30
40
50
60
70
80
90
100
110
120
PEAK HEIGHT (CHART UNITS)

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF MANEB IN WASTEWATER

METHOD 129

1. Scope and Application

1.1 This method covers the determination of maneb.

Parameter	Storet No.	CAS No.
Maneb	--	12427-38-2

1.2 This is a spectrophotometric method applicable to the determination of maneb in aqueous waste effluents.

2. Summary of Method

2.1 Maneb is determined in wastewater using a carbon disulfide evolution procedure. The carbon disulfide formed by the hydrolysis of maneb is measured colorimetrically after it is reacted with an alcoholic solution containing amines and cupric acetate to form cupric diethyldithiocarbamate.

2.2 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

2.3 The method detection limit (MDL) for maneb is 0.02 ug/g (ppm) based on a 1000-g sample.

3. Interferences

3.1 This method is not compound-specific since CS_2 or any compounds that are capable of hydrolyzing to CS_2 will analyze as maneb. If unusually or surprisingly high results are obtained, additional analytical studies will be necessary to identify the source and/or to determine maneb specifically.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Distilling flask, 500 mL

5.2.2 Decomposition Absorption Apparatus (See Figure 1)

5.3 Spectrophotometer, Beckman Model B or equivalent

6. Reagents

6.1 Color reagent -- To 0.012 g cupric acetate monohydrate in 250-mL volumetric flask, add 25 g diethanolamine. Dilute to volume with EtOH and mix.

6.2 NaOH solution -- 10%, w/v.

6.3 Carbon disulfide standard solution -- To accurately tared 25-mL volumetric flask containing 5 mL ethanol, pipet approximately 0.1 mL CS_2. Close flask at once and reweigh to obtain weight of CS_2 by difference. Dilute to mark with ethanol and mix well. Dilute 2 mL of this solution to 100 mL with ethanol and mix (standard solution). Calculate ug CS_2/mL.

6.4 Stannous chloride -- 40%, w/v.

7. Calibration

7.1 Determine the absorbance at 435 nm

7.2 Calibration Procedure

7.2.1 To series of 25-mL volumetric flasks, add amounts of standard CS_2 solution varying from 0.1 to 3 mL, using 10-mL buret. To each flask, add 15 mL color reagent. Dilute to mark with ethanol and mix. Let stand 15 minutes and read absorbances at 435 nm against mixture of 15 mL color reagent and 10 mL ethanol as reference. Plot absorbances against ug CS_2.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Transfer a 250-g aliquot of the sample into the 500-mL distilling flask of the Decomposition-Absorption apparatus described in Reference 15.1. Assemble the equipment, and using the reagents and procedure outlined in Reference 15.2, continue the analysis. At the end of the boiling period, cool the 500-mL distillation flask containing the 250-mL sample and replace it with a second 500-mL flask containing a second 250-mL aliquot of the sample. Do not change the absorption towers. Collect the evolved CS_2 from the second run in the same color reagent trap. Continue to analyze the sample using a third and a fourth 250-mL aliquot and collecting the evolved CS_2 from all 4 runs in the sample trap.

The total sample weight is 1000 g. It is not possible to analyze a 1000-g single sample with the existing equipment because of handling difficulties, i.e. pressure build-up. Scaled-up equipment can be used, however, if desired.

10.2 At the conclusion of the fourth run, drain the CS_2 trap contents into a 250-mL volumetric flask, rinse with a small volume of ethyl alcohol, dilute to bring to volume with ethyl alcohol, and mix thoroughly.

11. Cleanup and Separation

Not Available.

12. Sample Analysis

12.1 Determine the absorbance at 435 nm of the solution in Step 10.2 and calculate the amount of maneb in ppm using a calibration curve previously prepared using analytical standard maneb.

13. Calculations

13.1 Calculate ug dithiocarbamate from equivalent CS_2 found. If the dithiocarbamate(s) present is unknown, calculate as maneb by following formula:

$$\text{ug Maneb} = \text{ug } CS_2 \times \frac{\text{maneb (g molecular weight)}}{2 \times CS_2 \text{ (g molecular weight)}}$$

$$= \text{ug } CS_2 \times 1.81$$

14. Method Performance

14.1 The method has a sensitivity of 0.02 ppm using a 1000-g sample.

15. References

15.1 Determination of Dithiocarbamate Fungicide Residues, by H.L. Pease, JAOAC, 40, 1113 (1957).

15.2 Collaborative Study of the Determination of Dithiocarbamate Residues by a Modified Carbon Disulfide Evolution Method by George E. Keppel, JAOAC, 54, 528 (1971).

15.3 "Determination of Maneb in Plant Aqueous Waste Effluent," Standard Test Method, DuPont Company, Laporte, TX.

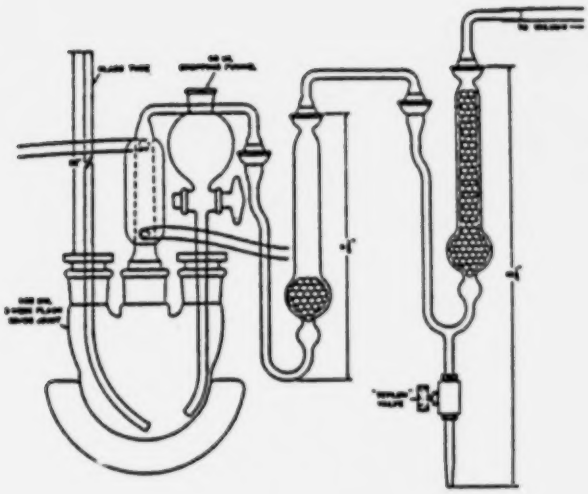


FIGURE 1.--Decomposition-absorption apparatus for dithiocarbamate determinations.

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United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
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Water and Waste Management

TEST METHOD

DETERMINATION OF MEPHOSFOLAN [CYTROLANE(TM)], PHORATE [THIMET(TM)], AND TERBUFOS [COUNTER(TM)] IN WASTEWATER

METHOD 130

1. Scope and Application

1.1 This method covers the determination of certain organophosphorus compounds. The following parameters can be determined by this method.

Parameter	Storet No.	CAS No.
Mephosfolan (CYTROLANE)	--	950-10-7
Phorate (THIMET)	--	298-02-2
Terbufos (COUNTER)	82088	13071-79-9
Malathion	39530	121-75-5
Dimethoate (CYGON)	--	60-51-5
Famphur	--	--

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in process wastewater streams.

1.3 The method detection limit (MDL) for the pesticides covered by this method is in the range of 5 to 25 mg/L (based on the original waste sample) when a flame ionization detector is employed, and 5-25 ug/L when an alkali flame ionization detector is employed.

2. Summary of Method

2.1 A measured volume of sample (1-liter)is extracted with several portions of chloroform. The combined chloroform extracts are concentrated and analyzed by gas chromatography with flame ionization and/or alkali flame ionization detector.

3. Interferences

3.1 All solvents, reagents, glassware, sample handling equipment, and instrumentation must be demonstrated to be free from pesticides and/or artifacts which could cause interference or misinterpretation of gas chromatograms.

3.2 Some organic compounds present in mixed plant waste streams cause interferences in the analysis of organophosphorus pesticides by gas chromatography, using a flame ionization detector. These interferences can often be overcome by the use of an alkali flame (phosphorus specific) ionization detector.

4. Safety

4.1 All normal laboratory safety equipment and procedures should be used.

4.2 Personal protective clothing should be worn, including safety glasses, laboratory coat, and rubber gloves.

4.3 All solvent handling should be performed in a well ventilated laboratory hood.

4.4 All gas chromatographic work should be performed by an experienced analyst.

4.5 Some organophosphate pesticides are very toxic and must be handled carefully, e.g., phorate exhibits an L/D_{50} of approximately 1 mg/kg (rat oral).

4.6 If solution containing organophosphate pesticide is spilled onto the skin or splashed into the eyes, wash the affected area with tap water for a minimum of 5 minutes. In severe cases of exposure, seek medical attention. All contaminated clothing must be removed immediately.

4.7 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Microliter syringes (Series No. 700, Hamilton Company), 10 uL

5.2.2 Separatory funnels, 100 mL

5.2.3 Beakers, 150 mL

5.2.4 Watch glasses, Speedyvap Ace Scientific Cat. No. 14-9420

5.2.5 Volumetric flasks, (10 mL, 25 mL, 50 mL, 100 mL and 200 mL)

5.2.6 Graduated cylinders (25 mL, 100 mL, 500 mL)

5.2.7 Glass wool, silane-treated (No. 14502, Applied Science Labs)

5.3 Gas Chromatograph -- An instrument designed for use with dual glass columns and equipped with an on-column injection system, and high-sensitivity flame ionization detectors or alkali flame ionization detectors and an electrometer having a sensitivity of at least 10^{-12} amperes driving a 1-mv strip chart recorder, and having a drift of less than 1% per hour (a totally solid state amplifier with an FET input) is recommended. Electronic digital integration or computer-based area measurements may also be employed. The integrator should have independent controls for the selection of up and down slope sensitivities so that start and stop integration points can be selected. An automated sample injection system also contributes significantly, particularly to analytical precision. The Hewlett-Packard Model 7600 and the Varian Model 2800 are suitable when equipped as described. Other equivalent instrumentation may be used but may require modification of operating conditions in order to obtain good peak shape, adequate resolution, and appropriate retention times.

5.3.1 Gas Chromatographic Column -- 122-cm borosilicate glass tube (4-mm I.D., 6-mm O.D.) bent to fit the chromatograph and packed with 3% OV-25 on Gas Chrom Q (100/120 mesh). A prepared packing can be purchased from Supelco, Inc., Supelco Park, Bellefonte, PA 16823, or Alltech Associates, 202 Campus Drive, Arlington Heights, IL 60004.

6. Reagents

6.1 Chloroform, reagent grade

6.2 Pesticide standards -- High purity pesticides: Malathion, THIMET, COUNTER, CYGON, CYTROLANE, and Famphur, may be obtained from American Cyanamid Company, Agricultural Division, Princeton, NJ 08540.

6.3 Di(2-ethylhexyl) sebacate: Applied Science Laboratories, Inc., State College, PA 16801.

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Gas chromatographic operating conditions are considered acceptable if the response to malathion is at least 50% of

full scale when ≤0.6 ug is injected for flame ionization detection and ≤0.6 ng is injected for alkali flame ionization detection. For all quantitative measurements, the detector must be operated within its linear response range.

7.2.2 Standards should be injected frequently to check the stability of the operating conditions.

7.2.3 Either external standardization or internal standardization methods may be used. When the internal standardization method is employed with flame ionization, di-(2-ethylhexyl) sebacate is used as the internal standard.

8. Quality Control

8.1 Duplicate sample injections should be used as quality control checks. In addition, known "standard" samples should be injected during each run as an additional check.

8.2 A method blank of distilled water should be extracted and carried through all analytical steps for each set of samples.

8.3 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Non-persistent pesticide compounds hydrolyze in water solutions; therefore, wastewater should be extracted immediately.

9.2 If it is necessary to store wastewater samples for even a short period of time, the samples should be refrigerated.

9.3 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Depending on the level of detection required in the waste sample, 200 to 600 mL of sample is required for analysis.

10.2 Mix the sample thoroughly, and quantitatively transfer the required amount to a 1000-mL separatory funnel.

10.3 Add 15 mL of chloroform to the sample in the separatory funnel and shake vigorously for two minutes.

10.4 Allow the solvent to separate from the sample, then drain the solvent phase into a 50-mL volumetric flask (if concentration is not required) or into a 150-mL beaker (if concentration is required).

10.5 Perform second and third extractions in the same manner.

10.6 Add a specific amount of di-(2-ethylhexyl) sebacate to the 50-mL volumetric flask if the internal standardization method is to be used, and fill the flask to the mark with chloroform.

10.7 Analyze the extract by gas chromatography.

10.8 The external standardization method is employed if a low detection limit is required. The chloroform extract is concentrated by covering the 150-mL beaker of extract with a watch glass and allowing the extract to evaporate to dryness in a well-ventilated hood overnight. Do not use heat to evaporate the extract because some pesticide compounds exhibit a high vapor pressure. In addition, uncontrolled heat may cause degradation of the non-persistent pesticides.

10.9 Carefully rinse the extract residue from the 150-mL beaker into a 10-mL volumetric flask with small portions of chloroform and fill the flask to the mark.

10.10 Analyze the concentrated extract by gas chromatography.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

13. Calculations

13.1 Determine the pesticides concentration by using either external standardization or internal standardization.

13.2 In the external standardization method, the gas chromatographic response of the sample extract is compared to the response of a pesticide standard.

13.2.1 The pesticides concentration of the sample is calculated as follows:

$$\text{micrograms/liter} = \frac{(Ai)}{(KF)\ (Vs)}$$

where Ai = Area of the pesticide component in the extract injected.

Vs = Volume (liters) of wastewater sample extracted.

KF = Calibration factor for the pesticide component.

$$KF = \frac{(Ac)}{(Cc)}$$

where Ac = Area of pesticide standard in the calibration standard injected.

Cc = Amount of the pesticide in the calibration standard (micrograms).

13.3 In the internal standardization method the gas chromatographic response of the sample extract is quantified by the addition of a known internal standard; this response is compared to response of a pesticide standard containing the same internal standard.

13.3.1 The pesticide concentration of the sample is calculated as follows:

$$\text{milligram/liter} = \frac{(IS)}{(Vs)} \frac{(KF)}{(Ais)} \frac{(Ai)}{}$$

where IS = Internal standard amount (milligrams)

Ai = Area of the pesticide component in extract injected

Vs = Volume of wastewater sample extracted (liters)

Ais = Area of the internal standard in the extract injected

KF = Calibration factor of the pesticide component

$$KF = \frac{(Cc)}{(A)} \frac{(Ais)}{(Cis)}$$

where Cc = Amount of pesticide component in the calibration sample (milligrams)

Cis = Amount of internal standard in the calibration sample (milligrams)

Ais = Area of the internal standard in the calibration sample

A = Area of the pesticide component in the calibration sample

13.4 Report results in milligrams per liter (mg/L) or micrograms per liter (ug/L) without correction for recovery data.

14. Method Performance

14.1 The detection limit for the pesticides covered by the method is

14.1 The detection limit for the pesticides covered by the method is in the range of 5 to 25 mg/L (based on the original waste sample) when a flame ionization detector is employed.

14.2 The detection limit for the pesticides covered by the method is in the range of 5 to 25 ug/L (based on the original waste sample) when an alkali flame ionization detector is employed.

15. References

15.1 Methods for Analysis of American Cyanamid Company.

15.2 Zweig, G & Sherma, J, Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, Gas Chromatographic Analysis, Academic Press, NY, 1972.

15.3 "Developmental Laboratory Procedure for the Detection of American Cyanamid Company Non-Persistent Organophosphorus Pesticides in Wastewater," Standard Test Method, American Cyanamid Company, Linden, NJ.

Table 1

Chromatographic Conditions

Instrumentation Operation Parameters

I. The following operating parameters are used with the Hewlett-Packard Model 7600:

Operation Mode	Dual Column
Detector	Flame Ionization
Column-oven, programmed temperature	130°C - 250°at 4°C/min
Injection port temperature	230°C
Detector temperature	270°C
Helium flowrate	75 mL/min
Hydrogen flowrate	50 mL/min
Air flowrate	500 mL/min
Range	10^3
Attenuation	1
Chart Speed	1/4 inch/min

II. The following operating parameters are used with the Varian Model 2800:

Operating Mode	Single Column
Detector	Alkali Flame Ionization
Column-oven, programmed temperature	170°C - 250°C at 8°C/min
Injection port temperature	230°C
Detector temperature	270°C
Helium flowrate	65 mL/min
Hydrogen flowrate	24 mL/min
Air flowrate	250 mL/min
Range	10^{-12}
Attenuation	Variable
Injection Volume	2 uL
Chart Speed	40 cm/hr

Table 2

Retention Times

Condition I

Parameter	Retention Time (min)
THIMET (phorate)	8.7
COUNTER (terbufos)	9.5
CYGON	13.3
MALATHION	16.7
CYTROLANE (mephosfolan)	22.5
Famphur	25.8
Internal Standard	28.3

Condition II

Parameter	Retention Time (min)
TIMET (phorate)	3.3
COUNTER (terbufos)	4.3
CYGON	6.7
Malathion	7.7
CYTROLANE(mephosfolan)	12.3
Famphur	13.3

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TEST METHOD

DETERMINATION OF METHAM [VAPAM(TM)] IN WASTEWATER

METHOD 131

1. Scope and Application

1.1 This method covers the determination of metham as its degradation product, methyl isothiocyanate.

Parameter	Storet No.	CAS No.
Metham	—	137-42-8

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater. This method assumes that complete degradation of metham to methyl isothiocyanate has occurred prior to analysis.

1.3 The method detection limit (MDL) for the degradation product, methyl isothiocyanate, is 2 ug/L, which corresponds to approximately 4 ug/L as metham. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A measured volume of water sample is extracted with chloroform or ethyl acetate. Sample volume is dependent on the extraction solvent used. Methyl isothiocyanate in the extract is determined by gas chromatography using a flame photometric detector in the sulfur mode.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnels, 250-mL and 1-liter with Teflon stopcocks.

5.2.2 Syringe, 10-uL Hamilton No. 701 or equivalent

5.3 Gas Chromatograph -- Hewlett Packard Model 5711A or equivalent, equipped with a flame photometric detector in the sulfur mode.

5.3.1 Gas Chromatographic Column -- 1.8-m x 2-mm I.D. Pyrex, packed with 100/120 mesh Supelcoport coated with 10% SP-2100, conditioned overnight at 235°C using a low nitrogen flow.

6. Reagents

6.1 Chloroform, nanograde or equivalent

6.2 Ethyl Acetate, nanograde or equivalent

6.3 Sodium Sulfate, anhydrous, reagent grade

6.4 Sodium Chloride, reagent grade

6.5 Methyl Isothiocyanate, available from Aldrich Chemical Co., Milwaukee, WI

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Prepare a stock solution of 1000 ug/mL methyl isothiocyanate in chloroform or ethyl acetate (see Step 10.2). From this solution, prepare a calibration solution to be used in the analysis.

7.2.2 Using the analytical conditions stated above, chromatograph 5 uL of a calibration solution of methyl isothiocyanate with a concentration approximately the same as that of the sample extract. Measure the peak height of the methyl isothiocyanate peak. Since the response of the flame

photometric detector in the sulfur mode is not linear, reference standard concentrations must be adjusted so that approximately equal peak heights are obtained when equal volumes of sample and reference solutions are analyzed.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Use either of the following procedures

10.1.1 Measure 50 mL of water sample (V_1) into a 4-oz bottle that contains 20 g dry NaCl. Pipet into the bottle 2.5 mL ethyl acetate. Cap the bottle with a foil-lined lid and shake it vigorously for 20 min. Remove the upper (ethyl acetate) phase via a disposable pipet and measure its volume (V_2). Dry the extract over anhydrous sodium sulfate.

10.1.2 Chloroform Solvent -- Measure 400 mL of water sample (V_1) into a 1-liter separatory funnel and extract twice with 10-mL portions of chloroform. Measure the volume of the combined extracts (V_2). Dry the combined extracts over anhydrous sodium sulfate.

10.2 Choice of solvent -- Either chloroform or ethyl acetate can be used. Use of ethyl acetate is preferable because it burns cleaner in the flame photometric detector. Use of chloroform can cause rapid fogging of the windows in this detector.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system daily as described in Section 7.

13. Calculations

13.1 Calculate the concentration of methyl isothiocyanate in water using the following equation:

$$ppm = \frac{H_s \times C \times V_2}{H_r \times V_1}$$

H_s = Peak height from sample, cm

H_r = Peak height from reference standard, cm

C = Concentration of methyl isothiocyanate in reference standard, ug/mL

V_1 = Volume of water sample, mL

V_2 = Total volume of chloroform or ethyl acetate extract, mL

14. Method Performance

14.1 Recoveries of methyl isothiocyanate from water are shown in Table 2. They are $\geq$ 80% at all fortification levels tested.

14.2 Using this method, the detection limit is 2 ug/L of methyl isothiocyanate. This level corresponds to approximately 4 ug/L of metham (Vapam).

15. References

15.1 "Determination of Methyl Isothiocyanate in Water by Gas Chromatography," Provisional Method, Stauffer Chemical Company, Westport, CT.

Table 1

Chromatographic Conditions

The instrument parameters listed below are for a Hewlett Packard Model 5711A gas chromatograph.

Injector temperature	200°C
Detector temperature	250°C
Column temperature	60°C
Carrier	N_2, 30 mL/min
Hydrogen	50 mL/min
Air	50 mL/min
Oxygen	0 mL/min
Volume injected	5 uL

Column: 1.8-m x 2-mm Pyrex packed with 100/120 mesh Supelcoport coated with 10% SP-2100.

Under these conditions, methyl isothiocyanate has a retention time of 3.5 min.

Table 2

Methyl Isothiocyanate Recoveries from Water

Solvent	Fortification Level, ug/L	Recovery, %
Chloroform	50	88
Ethyl acetate	10	83
Ethyl acetate	5	81
Ethyl acetate	2	85
	Average	84.3 ± 3.0

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TEST METHOD

DETERMINATION OF METHOMYL [NUDRIN(TM)] IN WASTEWATER

METHOD 132

1. Scope and Application

1.1 This method covers the determination of certain insecticides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Methomyl [NUDRIN(TM)]	39051	16752-77-5
Monocrotophos [AZODRIN(TM)]	81890	6923-22-4
Dicrotophos [BIDRIN(TM)]	—	141-66-2

1.2 This is a high-performance chromatographic (HPLC) method applicable to the determination of the compounds listed above in aqueous plant waste streams.

1.3 The method detection limit (MDL) for each of the parameters above is 0.1 mg/L (ppm). The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A measured volume of wastewater sample (250 mL) is adjusted to a pH of 6.5-8.0 and extracted with ethylene chloride. The extract is analyzed by two separate normal phase HPLC methods. The first method is used to ultimately determine the quantity of methomyl (NUDRIN) from the total of monocrotophos (AZODRIN) and methomyl (NUDRIN) insecticides and to indicate the presence of dicrotophos (BIDRIN)insecticide. If any of these insecticides are found to be present, the second method is employed to determine the monocrotophos (AZODRIN) and dicrotophos (BIDRIN) contents. The methomyl (NUDRIN) content is calculated as a difference between the total monocrotophos (AZODRIN) and methomyl (NUDRIN) found by the first method and the monocrotophos (AZODRIN) determined by

the second method. In all cases, the determinations are made by comparison of chromatographic peak heights with those obtained from standards of known concentration.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Volumetric glassware, including 50-mL, 25-mL, 10-mL, and 5-mL volumetric flasks; 15-mL pipet; and 250-mL and 1,000-mL graduated cylinders.

5.2.2 Separatory funnel, 500 mL.

5.2.3 Vacuum filtration apparatus, equipped with an 0.8-micron millipore membrane filter and a 500-mL vacuum flask.

5.2.4 Microsyringe, 50 uL.

5.2.5 Hydrometer, 1.000 to 1.200 specific gravity.

5.2.6 Round-bottom flask, 500-mL equipped with a reflux condensor.

5.3 Hot water bath, controlled at 90°C.

5.4 pH Meter

5.5 Liquid Chromatograph (1) capable of operating at 1,500 psi; (2) equipped with a variable wavelength UV detector capable of operating at 215 and 230 nanometers; (3) a loop injector valve with a 500-microliter loop; and (4) a 250-mm x 3.2-mm Partisil (Whatman), 10-micron PAC column.

6. Reagents

6.1 n-Heptane, distilled in glass, Burdick-Jackson or equivalent

6.2 Isopropanol, distilled in glass, Burdick-Jackson or equivalent

6.3 Tetrahydrofuran (THF), UV grade, distilled in glass, Burdick-Jackson or equivalent

6.4 Ethylene chloride (1,2-dichloroethane), distilled in glass, Burdick-Jackson or equivalent

6.5 Methanol, distilled in glass, Burdick-Jackson or equivalent

6.6 Sulfuric acid, 15%w

6.7 Glass wool

6.8 Sodium sulfate, anhydrous

6.9 (Monocrotophos) AZODRIN Insecticide (SD 9129) of known purity*

6.10 (Dicrotophos) BIDRIN Insecticide (SD 3562) of known purity*

6.11 (Methomyl) NUDRIN Insecticide (SD 14999-W) of known purity*

6.12 Sodium Hydroxide Solution, 50%w

* Available upon request from Shell Chemical Company, One Shell Plaza, HS&E Product Safety and Compliance, P.O. Box 4320, Houston, TX 77210

7. Calibration

7.1 Calibration Procedure

7.1.1 Weigh 25 mg (± 2 mg) to 0.1 mg of each of the insecticides into separate 5-mL volumetric flasks. Dilute to the mark with methanol. These are stock solutions of the standards.

7.1.2 Prepare a working solution containing monocrotophos (AZODRIN) and dicrotophos (BIDRIN) by carefully measuring 50-uL of these stock solutions into a 50-mL volumetric flask using a 50-uL microsyringe. Dilute to the mark with ethylene chloride. This solution contains approximately 0.25 mg each of monocrotophos (AZODRIN) and dicrotophos (BIDRIN).

7.1.3 Prepare a working solution of methomyl (NUDRIN) in the same manner as in Step 7.1.2.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Measure 250 mL of sample into a 500-mL separatory funnel.

10.2 Measure the specific gravity and check its pH. Adjust if necessary with 15%w sulfuric acid to a pH of 6.5 to 8.0.

10.3 Pipet 15 mL of ethylene chloride into the separatory funnel and shake vigorously for one minute. Allow phases to separate. If good separation occurs, skip to step 10.6. If poor separation occurs, empty and clean the separatory funnel and proceed with step 10.4, etc.

10.4 Filter greater than 250 mL of sample through an 0.8-micron membrane filter by vacuum filtration. Repeat step 10.3 with the filtered sample.

10.5 Withdraw the ethylene chloride phase into a 50-mL volumetric flask. If phase separation is still poor, the ethylene chloride phase should be passed through a small wad of glass wool in a funnel before entering the volumetric flask.

10.6 Repeat the extraction with two more 15-mL portions of ethylene chloride. (If glass wool was used, return it to the separatory funnel before the next extraction.)

10.7 Dilute the combined ethylene chloride extracts to 50 mL with ethylene chloride. If a large quantity of water is floating in the flask, remove it with a dropper and add sodium sulfate to dry the solution.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended HPLC operating conditions.

12.2 Instrument performance should be checked by injecting on the HPLC column 500 uL of the standard AZODRIN-BIDRIN mixture prepared in Step 7.1.2 under the chromatographic conditions (I) described in Table 1. Peaks should be present at 8.7 and 11.7 minutes.

12.3 For analysis, inject 500 uL of the sample extract prepared in Step 10.7 into the LC using chromatographic conditions (I) described in Table 1.

12.4 If no peaks appear at either 8.7 or 11.7 minutes, it may be assumed that neither AZODRIN, BIDRIN, nor NUDRIN is present at greater than 0.1 mg/L (ppm) in the sample (see Figures 1 and 2).

12.5 If a peak appears at 8.7 minutes in the sample, inject 500 uL of the NUDRIN standard solution prepared in Step 7.1.3 and record the NUDRIN peak.

12.6 Place a fresh 250-mL portion of the sample in a 500-mL round-bottom flask. Using the pH meter, add 50%w sodium hydroxide solution dropwise until a pH of >12 is reached.

12.7 Connect a reflux condensor to the flask and place it in a 90°C hot water bath for one hour. This will destroy AZODRIN and NUDRIN.

12.8 Cool and add 15%w sulfuric acid dropwise until a pH of 6-8 is reached.

12.9 Extract contents of flask with ethylene chloride as in Section 10.

12.10 Repeat step 12.3 with this extract. Mark chromatogram as "LAB HYDROLYZED." If the peak at 8.7 minutes remains the same as in the original extract, the peak was not due to NUDRIN or AZODRIN. If the peak at 8.7 minutes disappeared or was significantly reduced, determine the AZODRIN as follows.

12.11 Change the LC conditions to chromatographic conditions (II) as described in Table 1.

12.12 Rerun the original sample extract from Step 10.7 and the AZODRIN-BIDRIN mixture prepared in Step 7.1.2 (See Figures 3 and 4). If the peak appeared at 11.7 minutes in Step 12.4, BIDRIN is present and should be quantitated by using the conditions in step 12.12.

13. Calculation

13.1 To determine BIDRIN Insecticide, draw base lines for the 18.6-minute peak as shown in Figure 3 and 4. Measure the peak height of the sample and standard peaks. Calculate as follows:

$$\text{BIDRIN, ppm} = \frac{H_S \times C_{STD}}{H_{STD} \times 25 \times D \times E}$$

where H_S and H_{STD} = peak heights of the sample and standard, respectively

C_{STD} = concentration of insecticide in standard stock solution in mg/5 mL (Step 7.1.1)

D = sample density, g/mL

E = extraction coefficient = 0.5

13.2 To determine methomyl (AZODRIN) Insecticide, draw base lines for the 16.7-minute peak as shown in Figure 3 and 4. Measure the peak heights for the sample and standard peaks. Calculate as follows:

$$\text{(methomyl) AZODRIN, ppm} = \frac{H_S \times C_{STD}}{H_{STD} \times 25 \times D \times E}$$

where E, the extraction coefficient = 0.5

13.3 To determine NUDRIN Insecticide, draw a base line for the 8.7-minute peak of the chromatogram run at 230 nm as in Figure 1. Measure the peak height; this is due to AZODRIN and NUDRIN. The portion of this peak due to AZODRIN is obtained by multiplying the AZODRIN peak height for this sample, found in Step 13.2, by 1.08. This response factor is the ratio of peak heights for a given AZODRIN sample determined by the two methods. Subtract this value from the total peak height measured in this step to get the peak height due to NUDRIN.

13.4 Calculate the NUDRIN Insecticide as follows:

$$\text{NUDRIN, ppm} = \frac{H_{SC} \times C_{STD}}{H_{STD} \times 25 \times 4 \times 4}$$

where H_{SC} is the corrected sample peak height from step 13.3.

E is the extraction coefficient = 0.7

14. Method Performance

Not Available.

15. References

15.1 "Determination of NUDRIN, AZODRIN, and BUDRIN Insecticides in Aqueous Waste Streams by Liquid Chromatography," JH-LC-14A/80, Shell Chemical Company, Denver, CO.

Table 1

Chromatographic Conditions

I

Column		10-micron Partisil-PAC 250- x 3.2-mm
Mobile Phase		15% v/v isopropanol/heptane
Flow Rate		2 mL/min
Detection		UV at 230 nm
Retention Times:	methomyl (NUDRIN)	8.7 min
	monocrotophos (AZODRIN)	8.7 min
	dicrotophos (BIDRIN)	11.7 min

II

Column		10-micron Partisil-PAC 250- x 3.2-mm
Mobile Phase		5%/15%/80% volume ratio isopropanol/tetrahydrofuran/heptane
Flow Rate		2 mL/min
Detection		UV at 215 nm
Retention Times:	monocrotophos (AZODRIN)	16.7 min
	dicrotophos (BIDRIN)	18.6 min

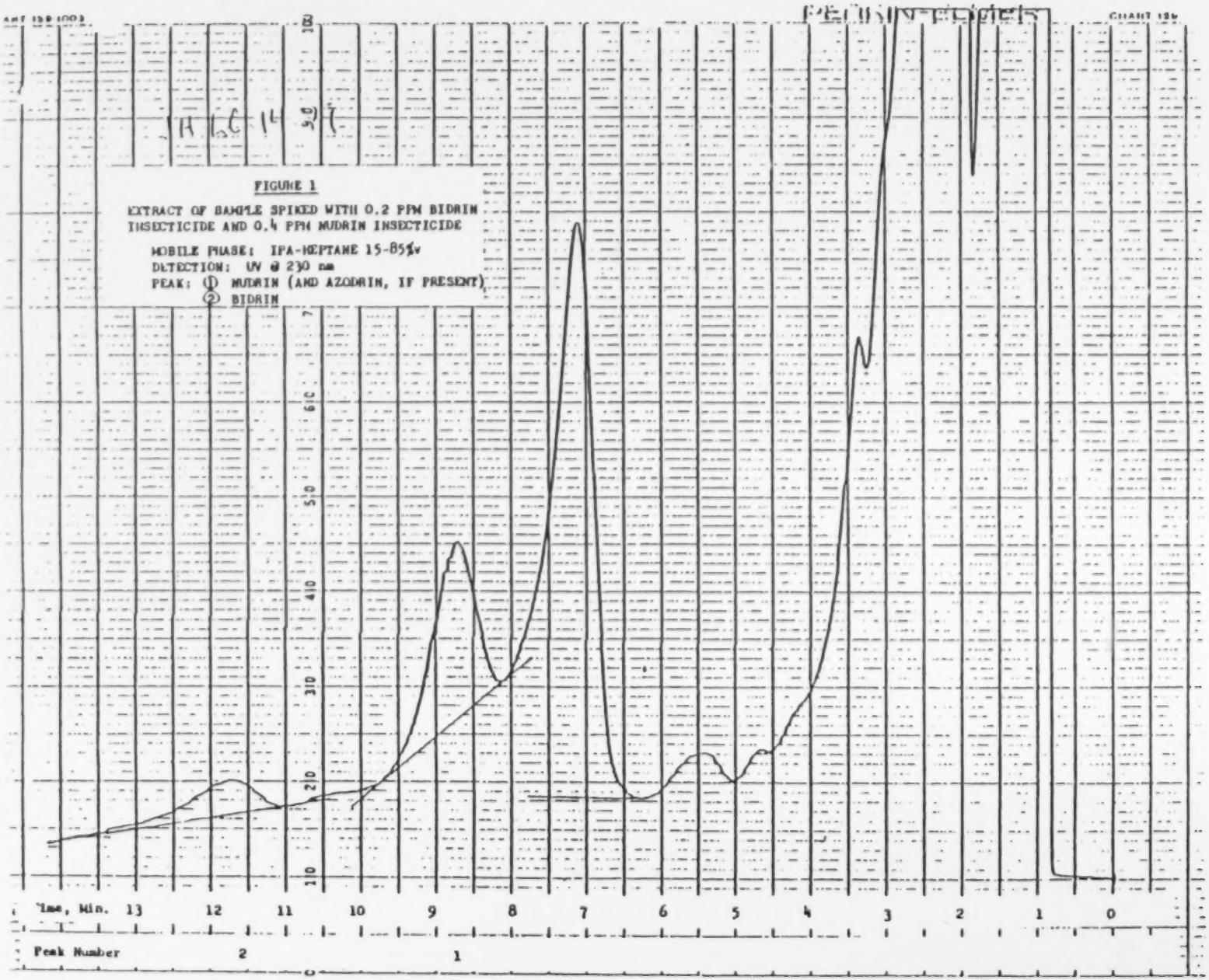
FIGURE 1
EXTRACT OF SAMPLE SPIKED WITH 0.2 PPM BIDRIN INSECTICIDE AND 0.4 PPM MUDRIN INSECTICIDE
MOBILE PHASE: IPA-HEPTANE 15-85%v
DETECTION: UV @ 230 nm
PEAK: ① MUDRIN (AND AZODRIN, IF PRESENT)
② BIDRIN
Time, Min. 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Peak Number 2 1

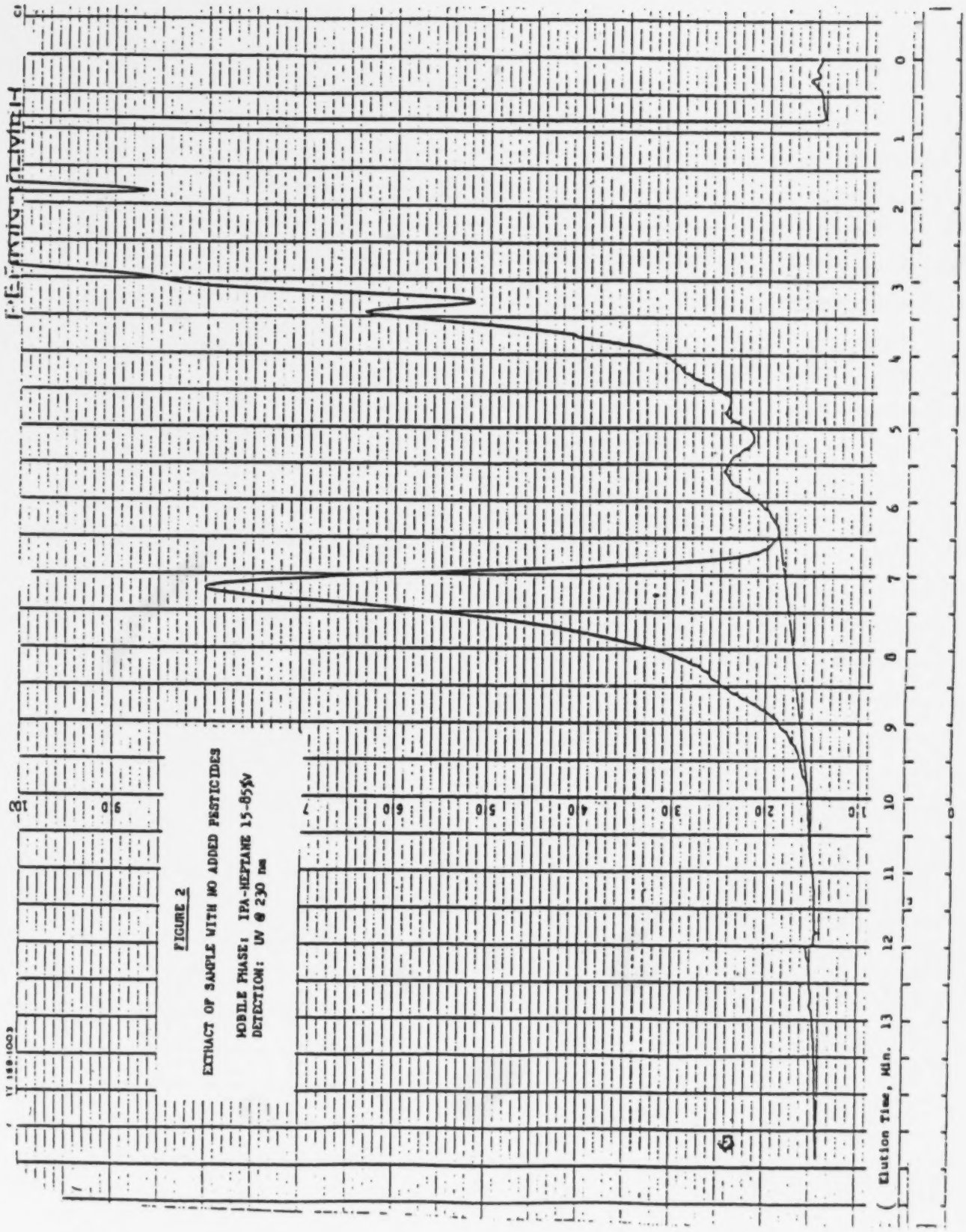

FIGURE 2

EXTRACT OF SAMPLE WITH NO ADDED PESTICIDES

MOBILE PHASE: IPA-HEPTANE 15-85%V
DETECTION: UV @ 230 nm

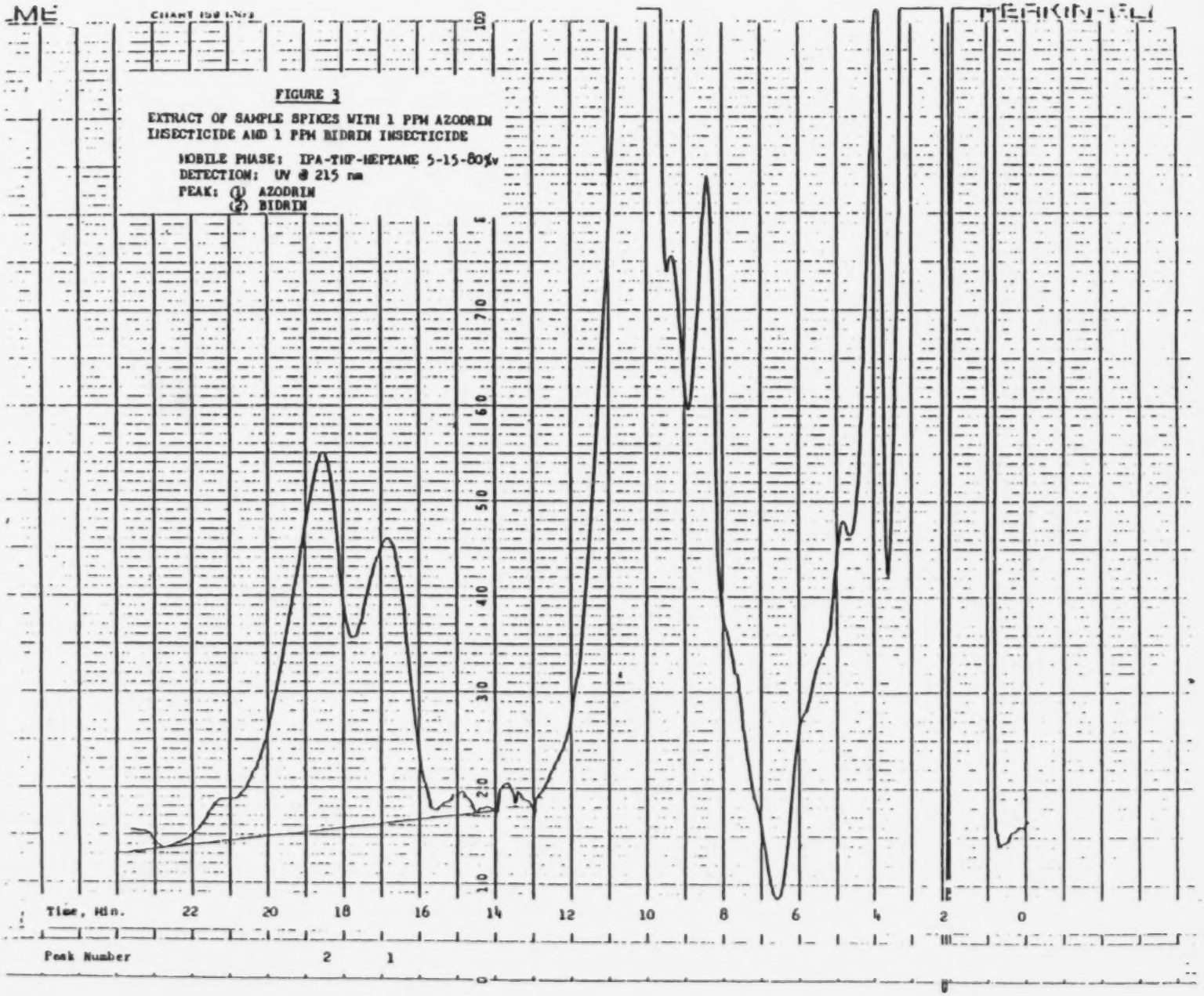

FIGURE 3

EXTRACT OF SAMPLE SPIKES WITH 1 PPM AZODRIN INSECTICIDE AND 1 PPM BIDRIN INSECTICIDE

MOBILE PHASE: IPA-THF-HEPTANE 5-15-80%v
DETECTION: UV @ 215 nm
PEAK: (1) AZODRIN
(2) BIDRIN

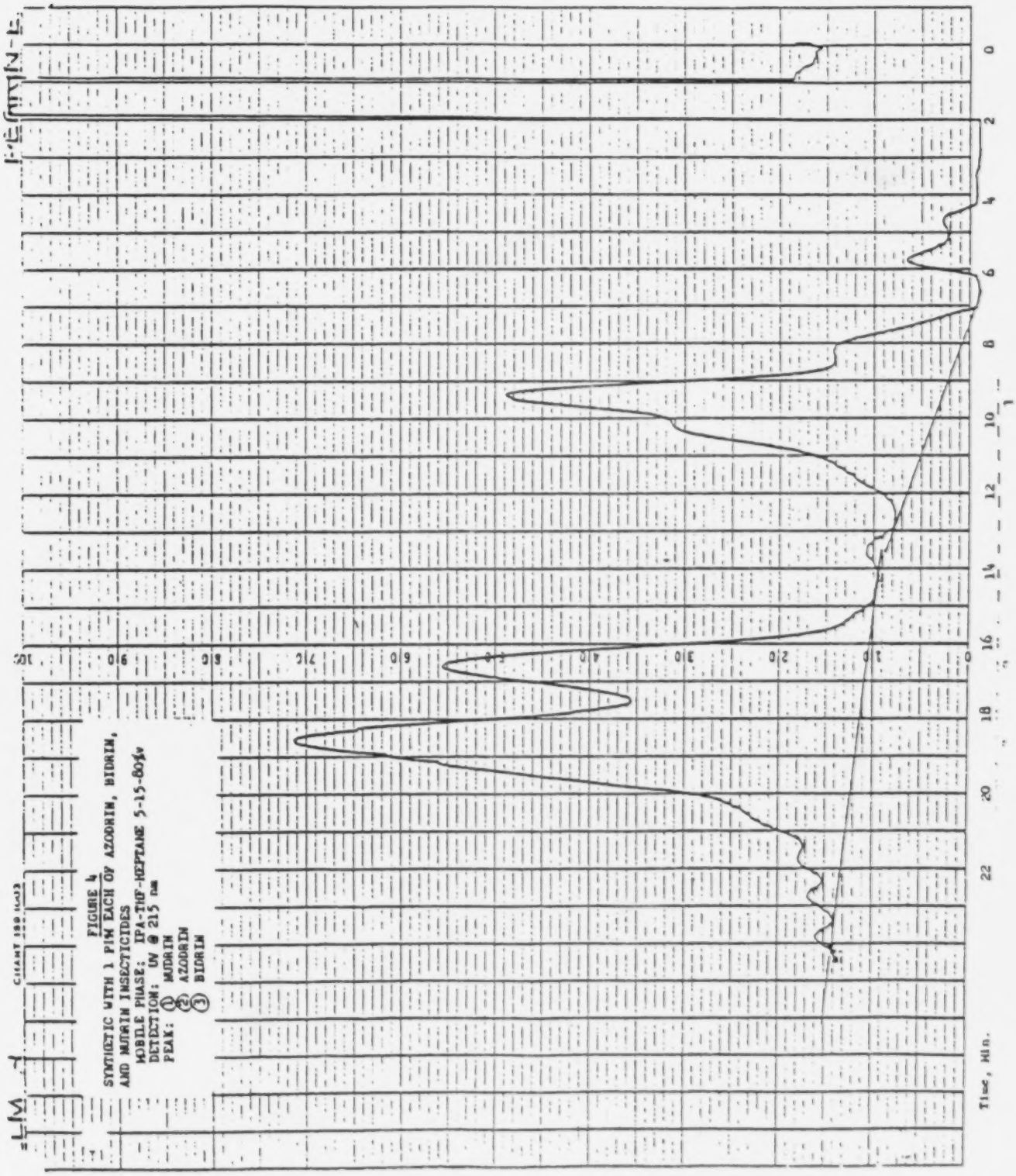

FIGURE 4
SYNTHETIC WITH 1 PIM EACH OF AZODRIN, BIDRIN, AND MODRIN INSECTICIDES
MOBILE PHASE: IPA-THF-HEPTANE 5-15-80%v
DETECTION: UV @ 215 nm
PEAK: ① MODRIN
② AZODRIN
③ BIDRIN

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TEST METHOD

DETERMINATION OF METHOMYL IN WASTEWATER

METHOD 133

1. Scope and Application

1.1 This method covers the determination of methomyl.

Parameter	Storet No.	CAS No.
Methomyl	39051	16752-77-5

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater.

1.3 The method detection limit (MDL) for methomyl is 0.01 ug/g (ppm) using a 100-g sample. The MDL for a specific wastewater may differ from that listed, depending upon the nature of interference in the sample matrix.

2. Summary of Method

2.1 A 100-g aliquot of wastewater (100 mL) is extracted with hexane. The hexane layer is discarded. The aqueous phase is hydrolized with NaOH, acidified, and extracted with ethyl acetate. The extract is dried and concentrated to a final volume of 1 mL by rotary evaporation. Analysis is by gas chromatography with flame photometric detector in the sulfur mode.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences.

3. Interferences

3.1 This method is not compound specific since an oximino compound, methyl N-hydroxythioacetimidate, or any compounds capable of hydrolyzing to methyl N-hydroxythioacetimidate will analyze as methomyl. If unusually or surprisingly high results are

obtained, additional analytical studies will be necessary to identify the source and/or to determine methomyl specifically.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnel, 250 mL

5.2.2 Beaker, 30 mL and 400 mL

5.2.3 Vacuum rotary evaporator

5.2.4 Volumetric flask, 1 mL

5.3 Steam bath

5.4 Gas Chromatograph with flame photometric detector

6. Reagents

6.1 Hexane

6.2 1N NaOH

6.3 1N H_2SO_4

6.4 Ethyl acetate

6.5 Triethylamine

7. Calibration

7.1 GC conditions

7.1.1 Establish chromatographic conditions as presented in references 15.1 and 15.2.

7.2 Calibration Procedure

7.2.1 Calibration procedures are given in references 15.1 and 15.2.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Transfer a 100-g sample into a 250-mL separatory funnel, add 50 mL of n-hexane, shake for 2 minutes, and allow the phases to separate. Discard the hexane layer. Repeat the hexane wash using a second 50-mL portion of n-hexane. Transfer the aqueous phase to a 400-mL beaker. Add 10 mL of 1N NaOH, cover, and heat on a steam bath for 20 minutes. Cool, acidify with 15 mL of 1N H_2SO_4, and quantitatively transfer the solution to a 250-mL separatory funnel using several small volumes of water as wash.

10.2 Extract the aqueous hydrolyzate with three 100-mL portions of ethyl acetate using 2-minute shaking periods for each extraction. Allow the phases to separate and filter the ethyl acetate phase through anhydrous sodium sulfate into a 500-mL round-bottomed flask.

10.3 Add 0.3 mL of triethylamine to the combined extracts and concentrate the solvent to about 10 mL using a vacuum rotary evaporator at 16°C. Quantitatively transfer the concentrated extract to a 30-mL beaker using several small volumes of ethyl acetate as wash. Add 0.1 mL more of triethylamine and carefully concentrate the solution to about 0.5 mL by evaporation at room temperature in a well-ventilated hood. [Do not allow the solvent to go to dryness.] Transfer the concentrated extract to a 1-mL volumetric flask using a fine-tip dropper and several small washes of ethyl acetate. Dilute to volume with ethyl acetate and mix thoroughly.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Equilibrate the gas chromatograph (the flame photometric detector is preferred) and inject an aliquot of the prepared extract as described in the reference methods (15.1 and 15.2). Measure the peak height of the oximino fragment and determine the micrograms of this material in the aliquot using a previously prepared calibration curve.

13. Calculations

13.1 Calculate the amount of methomyl present in ug/g (ppm) by dividing the micrograms found, corrected for the molecular weight conversion (1.54), and aliquot factors by the sample weight in grams.

14. Method Performance

14.1 The method has a sensitivity of 0.01 ug/g (ppm) using a 100-gram sample.

15. References

15.1 Determination of Methomyl Residue Using Microcoulometric Gas Chromatography by H.L. Pease and J.J. Kirkland, J. Agr. Food Chem., 16, 554 (1968).

15.2 Modification of the Published Gas Chromatographic Method for Determination of Methomyl Residues: Use of a Flame Photometric Detector by H.L. Pease, Unpublished, 1969.

15.3 "Determination of Methomyl in Plant Aqueous Waste Effluent," Standard Test Method, Vertac Chemical Corporation, Memphis, TN.

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TEST METHOD

DETERMINATION OF MEVINPHOS IN WASTEWATER

METHOD 134

1. Scope and Application

1.1 This method covers the determination of mevinphos.

Parameter	Storet No.	CAS No.
Mevinphos	39610	298-01-1

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater and plant effluent.

1.3 The method detection limit for mevinphos is 100 ug/L (ppb). The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A measured volume of water sample (100 mL) is diluted with saturated NaCl solution and extracted with isopropyl acetate. The extract is brought to 100 mL, dried, and analyzed by gas chromatography with electron capture detection.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Graduated cylinder, 500 mL

5.2.2 Assorted volumetric glassware and pipets as needed for samples and standards.

5.3 Gas Chromatograph

5.3.1 Hewlett Packard 5710A with electron capture detector, 2-ft x 2-mm glass column containing 10% EGSS-X coated on 100/120 mesh Chromosorb W-HP.

6. Reagents

6.1 Isopropyl acetate, nanograde

6.2 Sodium sulfate

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1

7.2 Calibration Procedure

Not Available.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 The amount of solution was measured and recorded. A 100-mL aliquot was placed in a 250-mL separatory funnel. Ten mL saturated NaCl solution was added, and 50 mL isopropyl acetate was added for extraction. The water layer was drained, and the isopropyl acetate layer was placed in a 100-mL glass-stoppered graduated cylinder. The water layer was then reextracted twice with 20 mL isopropyl acetate. The extracts were combined in the graduate. The extracts were brought to volume, and sufficient sodium sulfate was added to dry the solvent completely.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

13. Calculations

Not Available.

14. Method Performance

14.1 Recoveries were in excess of 90 percent.

14.2 Limits of detection 100 ug/L (ppb).

15. References

15.1 "Mevinphos in Waste Water," Standard Test Method, Amvac Chemical Corporation, Los Angeles, CA.

Table 1

Chromatographic Conditions

Column Temperature	150°C
Detector Temperature	300°C
Injector Temperature	200°C
Carrier/Flow Rate	N_2, 50 mL/min
Column: 2-ft x 2-mm glass, 10% EGSS-X coated on 100/120 mesh Chromosorb W-HP	
Retention Time (Mevinphos)	51 sec

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TEST METHOD

DETERMINATION OF PROFLURALIN IN WASTEWATER

METHOD 135

1. Scope and Application

1.1 This method covers the determination of profluralin.

Parameter	Storet No.	CAS No.
Profluralin	--	26399-36-0

1.2 This is a thin-layer chromatographic (TLC) method applicable to the determination of the compound listed above in wastewater.

1.3 The method detection limit (MDL) for profluralin is 6 mg/L (ppm). The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 An aliquot of sample (50 mL) is extracted with chloroform. The chloroform layer is isolated and used for spotting pre-coated TLC plates. The spots are eluted with benzene/chloroform/ethyl acetate (2/2/1). Detection is by air drying, exposure to chlorine gas, and spraying with a starch solution.

3. Interferences

Not Available.

4. Safety

4.1 Special precautions must be taken to avoid exposure to Cl_2 gas. Spraying must be done in a hood. The TWA for benzene is 1 ppm in

air and is a suspected human carcinogen. Precautions must be taken to preclude exposure to benzene vapors.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Capillary tubes -- Kimax 1.6, 1.8- x 100-mm

5.2.2 Chamber (25 cm x 29 cm x 10 cm)

5.2.3 Plates 200- x 200-mm glass

5.3 Plate: Silica Gel G, neutral (50% Merck Silica Gel G, 50% Bio Rad Bio Sil-A) on 200- x 200-mm glass plates coated at a thickness of 20 u.

6. Reagents

6.1 Silica Gel G, neutral (50% Merck Silica Gel G, 50% Bio Rad Bio Sil-A)

6.2 Chloroform

6.3 Profluralin Standard

6.4 Benzene

6.5 Ethyl alcohol

6.6 Starch

6.7 Potassium iodide

7. Calibration

7.1 Establish TLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 0.1000 g of profluralin of 99+% purity into a 100-mL volumetric flask. Dilute this stock standard to the mark with $CHCL_3$ and carry out a serial dilution to the fifth dilution. These solutions are equivalent to 200, 100, 50, 25, 12.5, and 6 mg/L when compared to samples extracted 5:1.

7.2.2 Fresh standard solutions should be prepared once per month.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Extract 50-mL samples with 10 mL $CHCl_3$ in a 125-mL separatory funnel. Isolate the $CHCl_3$ layer for spotting.

11. Cleanup and Separation

Not Available.

12. Thin Layer Chromatography

12.1 Normal principles of thin-layer chromatography apply for this analysis. Table 1 summarizes the recommended operating conditions for TLC.

12.2 Make one application using sample and standards using open-tip capillary tubes (7-8 uL), capillary tubes from Kimax (size 1.6, 1.8- x 100-mm).

12.3 After elution, air dry plate and expose to chlorine gas in a saturated chamber for 30 seconds. Remove plate and after 2 minutes spray with a starch solution prepared as follows:

12.3.1 Weigh 1.5 g starch into a beaker and add 50 mL water.

12.3.2 Weigh 0.5 g potassium iodide into another beaker and add 50 mL water.

12.3.3 Measure 20 mL 3A alcohol.

12.3.4 Heat solution A until starch goes into solution, then add KI solution and alcohol.

13. Calculations

Not Available.

14. Method Performance

14.1 The standard deviation of an analysis is approximately 30% relative to actual concentration.

14.2 A statement of "not detected" (ND) for profluralin indicates only that the profluralin content is less than 6 ppm.

15. References

15.1 "TLC Determination of Profluralin in Wastewater," Method No. ETM-154-1, June 6, 1977, Ciba-Geigy, AL.

Table 1

Chromatographic Conditions

Eluent	Benzene/chloroform/ethylacetate 2/2/1 (by volume)
Chamber:	Supersaturated (filter paper lining) Chamber size - 25 cm x 29 cm x 10 cm
Plate:	Silica Gel G, neutral (50% Merck Silica Gel G, 50% Bio Rad Bio-Sil-A), 200-mm x 200-mm, 20-micron
Temperature	Ambient
Distance	15 cm
Time	35 minutes
Rf (profluralin)	0.7
Point of Origin (Rf)	0.0

Note: A fresh eluent should be prepared each day and should be allowed to equilibrate 1 hour before use.

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TEST METHOD

DETERMINATION OF SIMETRYN IN WASTEWATER

METHOD 136

1. Scope and Application

1.1 This method covers the determination of triazine pesticides. The following parameters can be determined by this method.

Parameter	Storet No.	CAS No.
Simetryn	39054	1014-70-6
Prometryn	39057	7287-19-6
Ametryn	--	834-12-8

1.2 This is a thin-layer chromatographic (TLC) method applicable to the determination of the compounds listed above in wastewater.

1.3 The method detection limit (MDL) for each of the paramaters is 1 mg/L (ppm). The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 An aliquot of sample (50 mL) is extracted with chloroform. The chloroform layer is isolated and used for spotting on a pre-coated silica gel plate. Elution is with benzene/chloroform/ethyl acetate (2/2/1). Detection is by air drying, exposure to chlorine gas, and spraying with a starch solution.

3. Interferences

Not Available.

4. Safety

4.1 Special precautions must be taken to avoid exposure to Cl_2 gas.

Plate spraying must be done in a hood. The time weighted average for benzene is 1 ppm in air and benzene is a suspected human carcinogen. Precautions must be taken to preclude exposure to benzene vapors.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnel, 125 mL

5.2.2 Chamber (25 cm x 29 cm x 10 cm)

5.2.3 Capillary tubes (Kimax 1.6 - 1.8 x 100 mm)

5.2.4 Glass plates (200 x 200 mm)

5.3 Plate: Silica Gel G, neutral (50% Merck Silica Gel G, 50% Bio Rad Bio Sil-A) on 200- x 200-mm glass plates coated at a thickness of 200 u.

6. Reagents

6.1 Chloroform

6.2 Silica Gel G, neutral (50% Merck Silica Gel G, 50% Bio Rad Bio Sil-A)

6.3 Prometryn, Ametryn, and Simetryn Standards

6.4 Benzene

6.5 Ethyl Acetate

7. Calibration

7.1 Establish TLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 0.0160 g each of prometryn, ametryn, and simetryn of 99+% purity into a 100-mL volumetric flask. Dilute this stock standard to the mark with $CHCl_3$ and carry out a serial dilution to the fifth dilution. These solutions are

7.2.2 Fresh standard solutions should be prepared once per month.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly extract 50 mL water sample with 10 mL $CHCl_3$ in a 125-mL separatory funnel. Isolate the $CHCl_3$ layer for spotting.

11. Cleanup and Separation

Not Available.

12. Thin Layer Chromatography

12.1 Table 1 summarizes the recommended operating conditions of the TLC.

12.2 Prepare calibration standards as discussed in Section 7.

12.3 Make one application of samples and standards using open-tip capillary tubes (7-8 u), using capillary tubes from Kimax (size 1.6-1.8 x 100 mm).

12.4 After elution, air dry plate and expose to chlorine gas in a saturated chamber for 30 seconds. Remove plate and after 2 minutes spray with a starch solution prepared as follows:

12.4.1 Weigh 1.5 g starch into a beaker and add 50 mL water.

12.4.2 Weigh 0.5 g potassium iodide into another beaker and add 50 mL water.

12.4.3 Measure 20 mL 3A alcohol.

12.4.4 Heat solution A until starch goes into solution; then add KI solution and alcohol.

13. Calculations

Not Available.

14. Method Performance

14.1 A statement of "not detected" (ND) for a component indicates only that the component is less than 1 ppm.

14.2 The standard deviation of an anlaysis is 30% relative to actual concentration.

15. References

15.1 "TLC determination of Prometryn, Ametryn, and Simetryn in Wastewater," Method No. ETM-51-1, June 6, 1977, Ciba-Geigy, AL.

Table 1

Chromatographic Conditions

Eluent	Benzene/chloroform/ethylacetate 2/2/1 (by volume)
Chamber	Supersaturated (filter paper lining) Chamber size - 25 cm x 29 cm x 10 cm
Temperature	Ambient
Distance	15 cm
Time	35 min
Rf (simetryn)	0.2
Rf (ametryn)	0.3
Rf (prometryn)	0.4
Rf (point of origin)	0.0

Note: A fresh eluent should be prepared each day and should be allowed to equilibrate 1 hour before use.

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TEST METHOD

DETERMINATION OF TRIADIMEFON [BAYLETON(TM)] IN WASTEWATER

METHOD 137

1. Scope and Application

1.1 This method covers the determination of triadimefon.

Parameter	Storet No.	CAS No.
Triadimefon (BAYLETON[TM])	—	43121-43-3

1.2 This is a thin-layer chromatographic (TLC) method applicable to the determination of the compound listed above in wastewater.

2. Summary of Method

2.1 A measured volume of water sample (25 mL) is extracted with chloroform. The chloroform layer is spotted on pre-coated silica gel plates. The chromatogram is developed with toluene/ethyl acetate (9/1). The plate is sprayed with 1N methanolic sodium hydroxide and later with p-nitrobenzene diazonium fluoborate (NBDF), 0.2% methanol-water 1:1. The plate is then immersed in brown bromine fumes. Triadimefon is estimated by visual comparison and known standards spots.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Syringes, 10 uL and 50 uL

5.2.2 Plates, silica gel, E. Merck F0254

5.2.3 Funnel, separatory, 60-mL

5.3 Heat gun

5.4 Oven, 100°C

5.5 Tank, bromine

5.6 Tank, chromatographic

5.7 Spray bottle

6. Reagents

6.1 Triadimefon (BAYLETON) standard, 0.1% in acetone

6.2 Bromine, elemental

6.3 Chloroform, ACS

6.4 Methanolic sodium hydroxide, 1N -- dissolve 4 g of ACS sodium hydroxide pellets in 100 mL of methanol

6.5 Mobile solvent, toluene-ethyl acetate 9:1

6.6 p-Nitrobenzenediazonium fluoborate (NBDF), 0.2% in methanol-water 1:1 -- prepare fresh before use

7. Calibration

7.1 Establish TLC operating conditions equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Apply 10-, 25-, 50-, and 100-uL portions of the chloroform extract and the following amounts of standard to an E. Merck F-254 silica gel plate. Use a heat gun on the cool setting to keep the spots small.

0.1% BAYLETON mL	Corresponding BAYLETON, ppm, in Sample 10 mL	25 mL	50 mL	100 mL
1.0	20	8	4	2
2.5	50	20	10	5
5.0	100	40	20	10
7.5	150	60	30	15
10.0	200	80	40	20

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Pipet 25 mL of the aqueous sample and 5 mL of chloroform into a 60-mL separatory funnel.

10.2 Stopper the funnel and shake vigorously for 3 minutes.

10.3 Allow the layers to separate; then drain the chloroform layer into a 5-mL glass-stoppered graduated cylinder.

11. Separation and Cleanup

Not Available.

12. Thin Layer Chromatography

12.1 Develop the chromatogram with toluene-ethyl acetate 9:1 mobile solvent until the front is near the top.

12.2 Remove the plate and allow it to dry in a hood.

12.3 Spray the plate with 1N methanolic sodium hydroxide and allow the plate to air dry for 15 minutes.

12.4 Spray the plate heavily with freshly prepared NBDF and allow the plate to sit for 5 minutes.

12.5 Immerse the plate in brown bromine fumes.

12.6 Respray the plate with 1N methanolic sodium hydroxide.

12.7 Respray the plate with NBDF, then heat it at 110°C for 10 minutes. Triadimefon (BAYLETON) appears as reddish spots at approximately Rf 0.1.

13. Calculations

13.1 Estimate the BAYLETON in the sample by visual comparison with the known standard spots.

13.2 If the BAYLETON content in the sample is higher than the highest standard, dilute a portion of the chloroform extract from Step 10.3 at a 1:10 ratio with chloroform and repeat the test. In this case, multiply the apparent content by 10.

14. Method Performance

Not Available.

15. References

15.1 "Determination of Bayleton(R) in Waste Water," Analytical Method TM B-34,45, Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO.

Table 1

Chromatographic Conditions

Eluent	Toluene/ethyl acetate 9/1 (by volume)
Plate	Silica-gel, E. Merck F-254
Rf (triadimefon)	0.1

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TEST METHOD

DETERMINATION OF TRICHLORONATE [AGRITOX(TM)] IN WASTEWATER

METHOD 138

1. Application and Scope

1.1 This method covers the determination of trichloronate.

Parameter	Storet No.	CAS No.
Trichloronate [AGRITOX(TM)]	—	327-98-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater samples containing 0.005 - 1 ppm trichloronate.

2. Summary of Method

2.1 A measured volume of water sample (250 mL) is adjusted to pH 6.5 - 7.0 and extracted with chloroform. The extract is partitioned with distilled water, dried, and rotary evaporated to dryness. The residue is dissolved in acetone and reacted with potassium permanganate. The mixture is diluted with water and extracted with chloroform. The extract is then partitioned with distilled water, dried, and rotary evaporated to dryness. The residue is dissolved in acetone and analyzed by gas chromatography using a phosphorus (thermionic) detector.

3. Interferences

3.1 All glassware used for analyzing wastewater samples must be prerinsed with chloroform and not previously used for any analysis other than wastewater. DO NOT USE PLASTIC WASH BOTTLES FOR ANY SOLVENT.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Microsyringe, 10-uL

5.2.2 Rotoevaporator, Buchi or equivalent

5.3 Water bath, 50-55°C

5.4 Gas chromatograph, Varian 1440 or equivalent, equipped with a phosphorus detector (rubidium sulfate salt pellet), a 3-ft x 1/8-inch O.D. glass column packed with 3% OV-225 on Gas Chrom Q 80/100 mesh, and glass injector and detector inserts. (If the column ends are long enough to be used as inserts, the glass inserts are not necessary.) Note: Use only Teflon-backed septa, Supelco No. 2-0459.

6. Reagents

6.1 Acetone, nanograde

6.2 Trichloronate (AGRITOX) standard, technical purity (82-85%)

6.3 Chloroform, nanograde

6.4 Hydrochloric acid, 1N -- Carefully add 21 mL of concentrated hydrochloric acid into a 25-mL glass-stoppered graduated cylinder containing approximately 200 mL of distilled water. Dilute to 250 mL with distilled water and mix thoroughly.

6.5 Magnesium sulfate, 20% -- Weigh 41 g of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) into a 100-mL glass-stoppered graduated cylinder. Add distilled water to the 100-mL mark, stopper, and mix.

6.6 Mineral oil solution, 2.5%-- Dilute 25 mL of mineral oil, Fisher #O-120 or equivalent, to 100 mL with nanograde chloroform.

6.7 Potassium permanganate, 0.M -- Weigh 1.6 g of potassium permanganate, ACS into a 10-mL glass-stoppered graduated cylinder. Dilute to 100 mlwith distilled water and mix thoroughly.

6.8 Sodium hydroxide, 1N - TM-28.3

6.9 Sodium sulfate, anhydrous, ACS

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 AGRITOX standard solution, 0.10% — Weigh 0.120 - 0.130 g of technical AGRITOX (82 - 85% purity) into a 100-mL glass-stoppered graduated cylinder. Dilute to volume with acetone, stopper, and mix thoroughly. This solution may be used for one week only.

7.2.2 Pipet a 1-mL aliquot of the 0.10% AGRITOX solution into a 100-mL volumetric flask, dilute to volume with acetone, and mix thoroughly. Label this solution "10 ng." Prepare fresh daily.

7.2.3 Pipet 5-, 10- and 20-mL aliquots of the 10-ng standard from Step 7.2.2 into separate 100-mL volumetric flasks. Dilute to volume with acetone and mix. Label these solutions 500, 1000, and 2000 pg, respectively. Prepare fresh daily.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample by shaking, then proceed immediately to Step 10.2.

10.2 Measure 250 mL of the well-mixed sample into a 500-mL separatory funnel.

10.3 If the pH is below 6.5 or above 7.0, adjust it to 6.5 - 7.0 with 1N sodium hydroxide or 1N hydrochloric acid using pH indicator paper or a pH meter.

10.4 Extract three times by vigorously shaking for 1 minute each time with fresh 50-mL portions of nanograde chloroform. Collect the three chloroform extracts in another 250-mL separatory funnel. If an emulsion forms, centrifuge the emulsified layer and add the clear chloroform layer obtained by centrifuging to the second separatory funnel. Return the water layer to the first separatory funnel.

10.5 Add 50 mL of distilled water to the combined chloroform extracts in the second separatory funnel and shake for one minute.

10.6 Filter the chloroform layer through a funnel containing 4 to 5 g of anhydrous sodium sulfate retained by a small glass-wool plug (pre-rinsed with 10 mL of chloroform), into a 300-mL 24/40 boiling flask. Rinse the sodium sulfate three times with 10 mL of chloroform, and add 10 mL of 2.5% mineral oil solution into the flask.

10.7 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.8 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.9 Add 5 mL of nanograde acetone into the sample flask. Swirl the flask to wash the inside wall with the acetone but do not allow the acetone to get on the joint or splash out.

10.10 Add 10 mL of the magnesium sulfate solution to the flask and swirl to mix.

10.11 Add 25 mL of 0.1M potassium permanganate solution and swirl. Allow the sample to stand for 30 minutes, making sure that there is an excess of potassium permanganate (as indicated by the purple color) the entire time. (Add the permanganate in 5-mL increments, if necessary, to maintain an excess; increase the standing time 5 minutes for each addition).

10.12 Quantitatively transfer the sample solution from Step 10.11 into a 500-mL separatory funnel, using five 40-mL portions of distilled water.

10.13 Add 50 mL of chloroform into the separatory funnel, stopper and shake vigorously for 1 minute.

10.14 Allow the layers to separate. Drain the chloroform layer into a clean 250-mL separatory funnel.

10.15 Repeat Steps 10.13 and 10.14 twice for total of 3 extractions.

10.16 Add 25 mL of distilled water to the chloroform extracts, stopper, and shake for 30 seconds.

10.17 Filter the chloroform layer through another funnel containing 4 to 5 g of anhydrous sodium sulfate and retained by a small glass-wool plug (pre-rinsed with 10 mL of chloroform) into a 300-mL boiling flask.

10.18 Rinse the sodium sulfate with three 10-mL portions of chloroform and combine with the chloroform extract.

10.19 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool to room temperature.

10.20 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.21 Pipet 5 mL of acetone into the flask from Step 10.20, stopper immediately, and rotate the flask so that the acetone washes down the inside of the flask. DO NOT allow the acetone to get on the neck or stopper.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Continue the analysis according to TM A-54.12 (Mobay reference method), programming immediately after each injection.

13. Calculations

Not Available.

14. Method Performance

Not Available.

15. References

15.1 "Determination of AGRITOX(R) in Waste Water by GLC," Analytical Method TM B-34, 48, Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, MO.

Table 1

Chromatographic Conditions

	Varian 1400
Attenuation	X32
Cell voltage	Flame
Detector temperature	230-240
Electrometer range	10^{-12}
Gas Flows:	
Carrier gas	He, 20 mL/min
Air	230 mL/min
Hydrogen	40 mL/min
Injector temperature	230-240°C
Oven temperature	
Initial	130°C
Final	240°C
Mode	Hold
Rate	15°C/min
Upper limit hold	15

Column: 3-ft x 1/8-in glass packed with 3% OV-225 on Gas Chrom Q 80/100 mesh.

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TEST METHOD

DETERMINATION OF TRICYCLAZOLE IN WASTEWATER

METHOD 139

1. Scope and Application

1.1 This method covers the determination of tricyclazole.

Parameter	Storet No.	CAS No.
Tricyclazole	--	41814-78-2

1.2 This is a high-pressure liquid chromatographic (HPLC) method applicable to the determination of the compound above in water.

1.3 The method detection limit (MDL) for tricyclazole is 20 ug/L using direct aqueous injection and 1 ug/L using solvent extraction. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 Water samples are filtered and injected directly into a high-pressure liquid chromatograph (HPLC). Alternatively, the water is acidified and extracted with dichloromethane. The extract is evaporated and the residue is dissolved in an appropriate volume of mobile phase (methanol/water, 50/50, v/v) for injection into the HPLC. Detection is accomplished with a UV absorbance detector at a wavelength of 254 nm.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.3 Analytical filter papers, folded, Schleicher and Schuell, 12.5 cm (No. 588) or equivalent

5.4 Magnetic stirrer and stirring bar

5.5 A high-pressure liquid chromatograph consisting of the following components (or equivalent models)

5.5.1 Waters Model 6000A Solvent Delivery System

5.5.2 Waters Model 440 Absorbance Detector (fixed wavelength, 254 nm)

5.5.3 Water Intelligent Sample Processor (WISP), Model 710A

5.5.4 Houston Instruments Omni Scribe strip chart recorder, 1-10 mv

6. Reagents

6.1 Solvents

6.1.1 Methanol, HPLC grade, distilled in glass

6.1.2 Water, HPLC grade, free of organic impurities

6.1.3 Dichloromethane, reagent grade, redistilled

6.2 Solutions

6.2.1 HPLC mobile phase -- methanol:water (50:50, v/v), filtered and degassed

6.2.2 Hydrochloric acid, concentrated

6.3 pH paper, range 1-14

6.4 Sodium sulfate, anhydrous, methanol washed

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Preparation of Standard Solutions

7.2.1.1 Standard Solution A (tricyclazole 1.0 mg/mL). Dissolve 100 mg of tricyclazole analytical standard in methanol in a 100-mL volumetric flask and dilute to volume.

7.2.1.2 Standard Solution B (tricyclazole 10.0 ug/mL). Transfer a 1.0-mL aliquot of Standard Solution A to a 100-mL volumetric flask and dilute to volume with HPLC quality water.

7.2.1.3 Standard Solution C (tricyclazole 1.0 ug/mL). Transfer a 10.0-mL aliquot of Standard Solution B to a 100-mL volumetric flask and dilute to volume with HPLC-grade water.

7.2.1.4 Standard Solution D (tricyclazole 0.10 ug/mL). Transfer a 10.0-mL aliquot of Standard Solution C to a 100-mL volumetric flask and dilute to volume with HPLC-grade water.

7.2.1.5 Standard Solution E (tricyclazole 1.0 ug/mL). Transfer a 10.0-mL aliquot of Standard Solution B to a 100-mL volumetric flask and dilute to volume with methanol:water (50:50).

8. Quality Control

8.1 Prepare duplicate recovery samples by fortifying two 200-mL aliquots of untreated water with 1.0 mL of Standard Solution C. Prepare these samples using the solvent extraction technique (Step 10.2) and analyze.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

Note: Samples may be prepared for analysis by one of the following techniques, depending upon the detection limit required for the assay.

10.1 Direct Injection Technique

10.1.1 Remove particulate matter from the sample by filtering an aliquot of water through folded filter paper into a 20mL screw cap vial, and transfer approximately 1mL of filtered water into a WISP

sample vial. (Alternatively, filter directly into the WISP sample vial.)

10.1.2 Cap the vial with a self-sealing septum cap and analyze the samples using the HPLC conditions listed in Table 1.

10.2 Solvent Extraction Technique

10.2.1 Transfer an aliquot of water (normally 200 mL) to a 250-mL separatory funnel. Acidify the water by adding 0.5 mL of concentrated hydrochloric acid, and check with pH paper to ensure that the pH of the water is 3 or less.

10.2.2 Extract the water by partitioning with three 20-mL aliquots of dichloromethane for at least 20 seconds each.

10.2.3 Combine the dichloromethane (lower phase) extracts by passing through a funnel containing sodium sulfate into a 250-mL evaporating flask.

10.2.4 Evaporate the dichloromethane just to dryness on a rotary vacuum evaporator with a 35°-45°C water bath.

10.2.5 Dissolve the residue in 2.0 mL of HPLC mobile phase (normally methanol:water, 50:50) and analyze the solutions using the HPLC conditions listed in Table 1.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph for both the direct injection and solvent extraction techniques.

12.2 Calibrate the system as discussed in Section 7.

12.3 Measure the HPLC peak height (or area) for tricyclazole in water samples using the instrumentation listed in Section 5 and the parameters on Table 1. (Note: The parameters listed below may be modified as needed to compensate for daily variations in instrument performance. Also, the injection volume and/or the absorbance sensitivity setting may be adjusted to keep peak responses "on scale" for a given set of samples. The parameters used for each set of samples should be recorded.)

13. Calculations

13.1 Calculations for Direct Injection Samples From Step 10.1

$$\text{mg/L (ppm) tricyclazole} = \frac{PR_{sa} \times C_{std}}{Pr_{std}}$$

where PR_{sa} = peak response (cm height or cm^2 area) for sample

PR_{std} = peak response for direct standard

C_{std} = concentration (ug/mL) of direct standard

13.2 Calculations for Extracted Samples from Step 10.2

13.2.1 $$\text{Percent Recovery} = \frac{\frac{PR_{rec}}{PR_{std}} \times C_{std} \times V_F \times 100 \text{ percent}}{\text{ug fortified}}$$

where PR_{rec} = peak response for recovery sample

VF = final volume (mL), including dilutions

13.2.2 $$\text{mg/L (ppm) tricyclazole} = \frac{\frac{PR_{sa}}{PR_{std}} \times C_{std} \times V_F \times 100 \text{ percent}}{V_I \times \text{Percent Recovery}}$$

where V_I = initial volume (mL) of water extracted

14. Method Performance

14.1 The method for the direct injection of water samples into the HPLC has a detection limit of approximately 0.020 mg/L of tricyclazole in water. If a lower detection limit (0.001 mg/L) is required for the assay, the dichloromethane extraction procedure should be employed. A summary of the recoveries obtained during validation of the two methods is presented on Table 2.

15. References

15.1 "Determination of Residue Levels of Tricyclazole in Water by High-Pressure Liquid Chromatography." Method No. AM-AA-CA-J037-AB-755, Eli Lilly and Company, Greenfield, IN.

Table 1

Chromatographic Conditions

<u>Parameters for Direct Injection (From Step 10.1)</u>

Precolumn	CO-PELL (ODS) or equivalent
Column	uBond pak C_{18} (10-uM particle size, 3.9-mm I.D. x 30 cm) or Lichrosorb RP-18 (10-uM particle size, 4.6-mm ID x 25 cm)
Mobile Phase	methanol:water (50:50, v/v)
Flow rate	0.8-1.5 mL/min (adjust to achieve the desired retention time)
Injection volume	1000 uL
Absorbance sensitivity	0.01-0.02 AUFS
Chart speed	0.25 cm/min
Direct standard	Standard Solution 9

<u>Parameters for Concentrated Extracts (From Step 10.2)</u>

Precolumn	same as above
Column	same as above
Mobile phase	same as above
Flow rate	same as above
Injection volume	200 uL
Absorbance sensitivity	0.02 AUFS
Chart Speed	same as above
Direct Standard	Standard Solution 8

Table 2

Summary of Fortified Sample Recoveries

Method	(mg/L) ppm Fortified	N	Percent Recovery (mean + s.d)
direct injection	0.020	6	101.6 ± 3.8
	0.200	6	91.5 ± 4.0
	2.000	6	94.3 ± 2.5
extraction	0.001	6	122.7 ± 31.4
	0.010	6	109.8 ± 2.8
	0.100	6	94.2 ± 3.3

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Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF GLYPHOSATE IN WASTEWATER

METHOD 140

1. Scope and Application

1.1 This method covers the determination of glyphosate.

Parameter	Storet No.	CAS No.
Glyphosate	39941	1071-83-6

1.2 This is a high-performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in industrial effluent.

1.3 The method detection limit (MDL) is 10 ug/L with 5% of full-scale deflection. The MDL for a specific wastewater may differ from that given, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A known volume of glyphosate industrial effluent is applied to a packed AG50W-X8 (400 mesh, H^+ form, Bio-Rad) cation exchange primary column via an auto injector. The selected fraction of column eluent is switched onto the analytical column. The Technicon Auto Analyzer-based ninhydrin post column reactor is employed as a detector.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Ammonia, as well as primary and secondary amines, will have a significant response with ninhydrin reagent, and, therefore,

prevent detection of the presence of the glyphosate peak in the chromatogram.

3.2 A sample clean-up using packed AG50W-X8 strong cation exchange primary column will eliminate the interferences of the above mentioned species. The regeneration of the primary column is feasible within the analysis time domain (40 min).

3.3 Equipment used in this analysis (columns, filters, glassware, etc.) should be limited to trace analysis only to eliminate any possibility of residual contamination at these low levels.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Pipets, 10-100 uL, 100-1,000 uL, and 1-5 mL delivery pipets with disposable tips.

5.2.2 Volumetric flasks, 100 mL or convenient sizes for making standards or dilution.

5.3 Two buffer pumps, Waters 6000A pump, flow rate 0.6 mL/min.

5.4 Injector, Waters Intelligent Sample Processor (WISP).

5.5 Technicon Proportionation Pump, Ninhydrin reagent reactor.

5.6 Post Column Reactor, Technicon research cartridge or oil bath controlled at $95^{\circ}C \pm 0.1^{\circ}C$.

5.7 Detector, Technicon Single Channel Colorimeter with 570-nm optical interference filter and 2.0-mm x 50-mm flow cell.

5.8 Electronic Filter, Spectrum 1021 filter and amplifier.

5.9 Recorder, Fisher, 100 mv, full scale.

5.10 HPLC Analytical Column, DuPont Zorbax SAX, 15 cm x 4.6 mm.

5.11 HPLC Primary Column, stainless steel tubing for LC, 15-cm x 4.0-mm I.D. (Handy & Harman Tube Co., Whitehall Road, Morristown, PA 19404).

5.12 Bed support, 1/4-in diameter, 15-u pore size (Whatman 4334-2351).

5.13 Bottom Drilled Swagelok fitting, P.J. Coert, 40061-BD.

5.14 Six-Part valve, air activated Valco valve (Model CF-6-UHPa-N60).

5.15 Digital Valve Sequence Programmer, DVSP, switching sequence controller.

5.16 Cation Exchange Resin for Primary Column, AG50W-X8, 400 mesh, H^+ form (Bio-Rad 142-1461).

5.17 CDC 1700 Computer System, Sample Injection, DVSP starter and data collection.

6. Reagents

6.1 Dimethylsulfoxide (Pierce, 20687).

6.2 Ninhydrin (Pierce, 21001).

6.3 Lithium Acetate pHix Buffer, 4M $Li^{\oplus}$; pH 5.20 (Pierce, 27203).

6.4 Hydrindantin Dihydrate (Pierce, 24000).

6.5 Methanol (MCB reagent, MX 0488).

6.6 H_3PO_4 (85%, Fisher Z-242).

6.7 KH_2PO_4 (Mallinckrodt, 7100).

6.8 Brij 35, 30% aqueous solution (Fisher, CS-285-2).

6.9 Argon

6.10 H_2SO_4 (Fisher, A-300).

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 A stock solution of 1,000 ug/mL (ppm) is prepared by dissolving 100 mg of 3X recrystallized analytical grade of glyphosate in 100 mL deionized water and stored in the refrigerator.

7.2.2 A series of working standards of 20, 40, 60, 80, and 100 ug/L (ppb) is prepared weekly by appropriate dilutions of 10 ug/mL (ppm) glyphosate standard solution, which is prepared from the dilution of 1,000 ug/mL (ppm) stock solution.

7.2.3 A series of external standards (20-100 ug/L) is prepared and analyzed under the same HPLC conditions and on the same day as unknown samples. A blank industrial effluent sample and water also have to be included to ensure the absence of glyphosate contamination due to incomplete regeneration of primary column during analysis.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Samples are filtered via syringe-type filter and ready for injection.

11. Cleanup and Separation

11.1 A sample clean-up using packed AG50W-X8 strong cation exchange primary column will eliminate the interferences of the above-mentioned species. The regeneration of the primary column is feasible within the analysis time domain (40 min).

11.2 The automated in situ sample clean-up via primary column essentially eliminates the possible contamination introduced due to sample manipulation (e.g., evaporation, etc.). The automatic method provides the minimum sample handling time so a larger number of samples can be analyzed.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

12.2 Calibrate the system as described in Section 7.

12.3 Primary Column Packing

12.3.1 Dimension--15-cm x 6.35-mm O.D. x 4.0-mm I.D.

12.3.2 Packing Material AG50W-X8, 400 mesh, H^+ form (Bio-Rad, 142-1461)

12.3.3 Packing Procedures

1) Prepare a slurry of AG50W-X8 in HPLC buffer solution (Step 8.2). Decant the free suspension and let stand overnight before application.

2) Cut 15 cm of stainless steel tubing. File both ends of tubing and sand to remove burrs.

3) Place bed support in end fitting; insert one end of column. Tighten nut and ferrule onto end fitting.

4) Fill column from open end with vacuum aspirator to settle packing material.

5) Attach end fitting with bed support, nut, and ferrule on open end.

6) Connect to LC pump and run buffer to settle packing.

7) Open column on inlet and check to see if there is a void. If there is, repeat steps 4-6 until packing material is packed tight and no voids remain in column.

12.4 Preparation of Reagent

12.4.1 HPLC Buffer Solution. Dissolve 10 g KH_2PO_4 in 3.8 L of 160 mL methanol/deionized water; adjust the solution to pH 2.3 with 85% H_3PO_4. Follow normal HPLC degassing/filtration procedures.

12.4.2 Ninhydrin Solution. Combine 1,100 mL of dimethylsulfoxide (DMSO), 800 mL of deionized water, 400 mL of 4.0M (pH 5.2) lithium acetate, and bubble argon through the solution for approximately 15 minutes. Add 32 g of ninhydrin to the solution with stirring. In a separate beaker dissolve 1.6 g of hydrindantin solution in two portions of 50 mL DMSO with a few mL of previously prepared ninhydrin solution; add together with argon bubbling. An additional 15 minutes for argon deoxygenation is required before the application.

12.4.3 Wetting Reagent for Autoanalyzer. Dissolve 10 mL Brij in 800 mL of deionized water; add a few drops of concentrated H_2SO_4 to make the solution acidic.

12.5 Construction of Ninhydrin Reactor

Refer to Figure 1.

12.6 Interfacing Between WISP and DVSP -- Refer to Figure 2.

12.7 Interfacing Between DVSP and Six Part Valve -- Refer to Figure 3. DVSP sequence timing is set as follows:

Sequence	Time (Minutes)
1	2.0
2	3.0

12.8 Interfacing Between Colorimeter and Spectrum -- Refer to Figure 4.

12.9 Configuration of Column Switching Instrumentation -- Refer to Figure 5.

12.10 HPLC Injection

12.10.1 An alternating injection between sample and standard is carried out through the analysis to ensure the complete regeneration of the primary column.

12.11 A typical chromatogram of 100 ug/L glyphosate in 002 sump synthetic waste is shown in Figure 6. The peak height of glyphosate standard can be optimized by adjusting switching time on DVSP as illustrated in Figure 7.

13. Calculations

13.1 The actual concentration of glyphosate in waste sample is

$$\text{ug/L (ppb)} = \frac{\text{(ug/L glyphosate from calibration curve)}}{\text{0.7 (spiking recovery)}}$$

13.2 The spiking recovery should be redetermined when a plant industrial effluent sample is available.

14. Method Performance

14.1 The method was validated over the range of 20-100 ug/L. The probable linear range is up to 10 mg/L with glyphosate standards.

14.2 The detection limit is 10 ug/L of glyphosate standard with 5% of full-scale deflection (S/N = 2).

14.3 The laboratory validation of this method was carried out using a synthetic glyphosate industrial waste sample. The components of the mixture are listed in Table 2.

14.4 The pooled coefficient of variation ($\overline{CV}$) for the analytical method in the range between 20 and 100 ug/L glyphosate in 002 sump synthetic waste is 0.0775.

14.5 The average values obtained for glyphosate in 002 sump synthetic waste are invariably 30% less than the "true value." The difference between the "found" and "true" concentrations is not due to a random variation of the experimentally determined "true" concentration but rather due to the actual constant sampling variation of glyphosate in the primary column. Therefore, the recovery should be applied to the final results in step 10.

15.1 "Automated Method for the Determination of Glyphosate in Industrial Effluents via Column Switching Technique," Standard Test Method, Monsanto Agricultural Products Co., St. Louis, MO.

Table 1

Chromatographic Conditions

HPLC Operation Conditions for Both Columns	
Buffer Flow Rate	0.6 mL/min
WISP Operation Parameters	
Injection Volume	1,000 uL
Run Time	25 minutes
Number of Injections	1
Detector	DAMP 2, Std. Cal. 1.00
Electronic Filter - Cutoff Frequency Attenuation	0.01 2.0
Recorder	Chart Speed 0.25 cm/min
Primary Column	15-cm x 4.0-mm AG 50W-X8, 200-400 mesh, H^+ form
Analytical Column	15-cm x 4.6-mm DuPont SAX
Mobile Phase	10g KH_2PO_4, 4% Methanol, pH = 2.3 with 85% H_3PO_4 dissolved in 4L of water

Table 2

The Composition of the Synthetic Glyphosate Industrial Waste Sample

Component	Composition (ppm)
Glyphosate Intermediate Raw Material	30
PO_4^{3-}	30
NH_3	100
NO_3^-	100
SO_4^{2-}	200
Cl^-	200
Na^+	200
Ca^{2+}	50
Mg^{2+}	10
Monoethanolamine	10

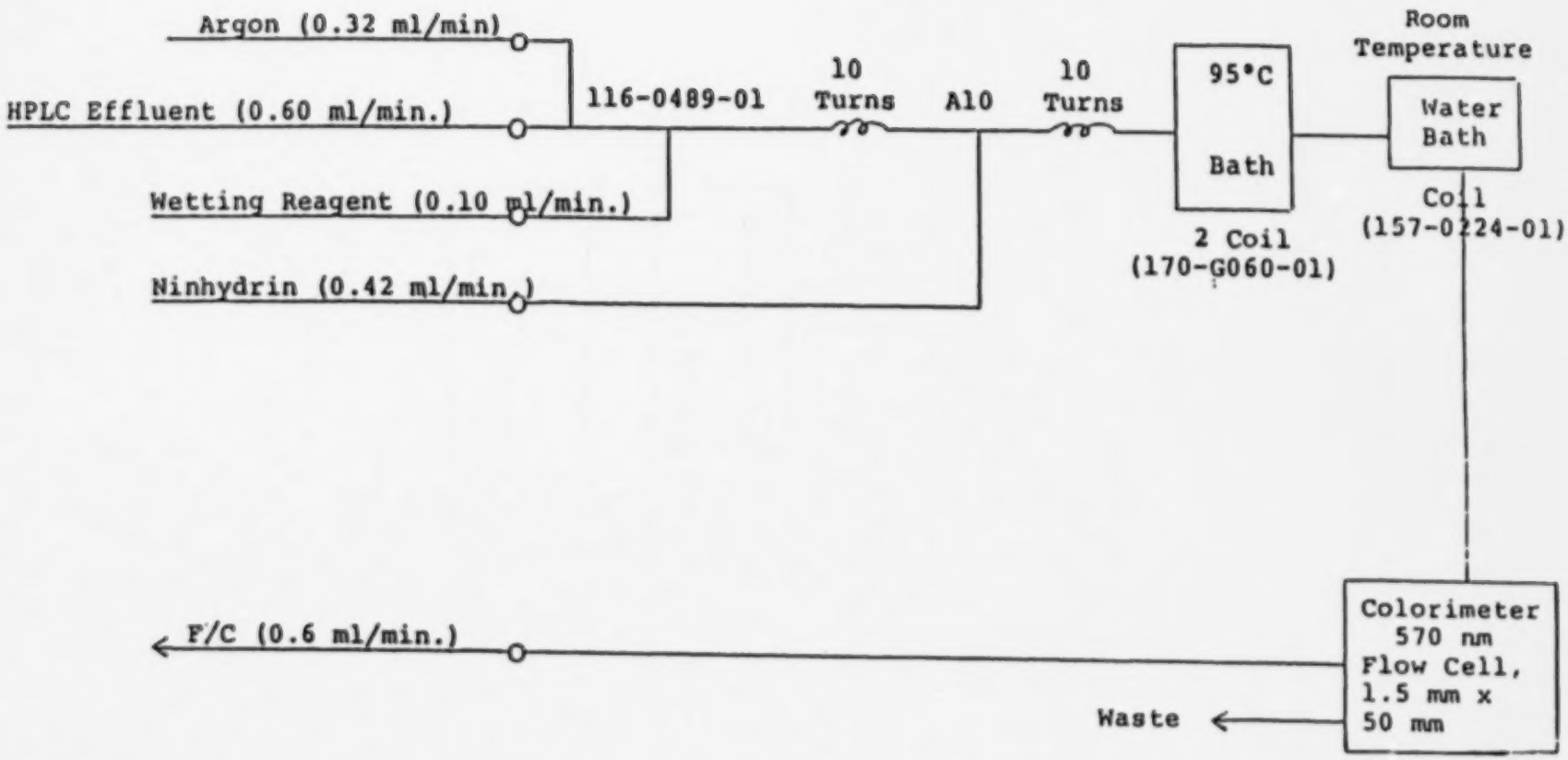


FIGURE 1.

The Hydraulic System of Technicon Autoanalyzer Based Ninhydrin Reactor

FIGURE 2.

Electrical Connections for Digital Valve Sequence Programmer and WISP

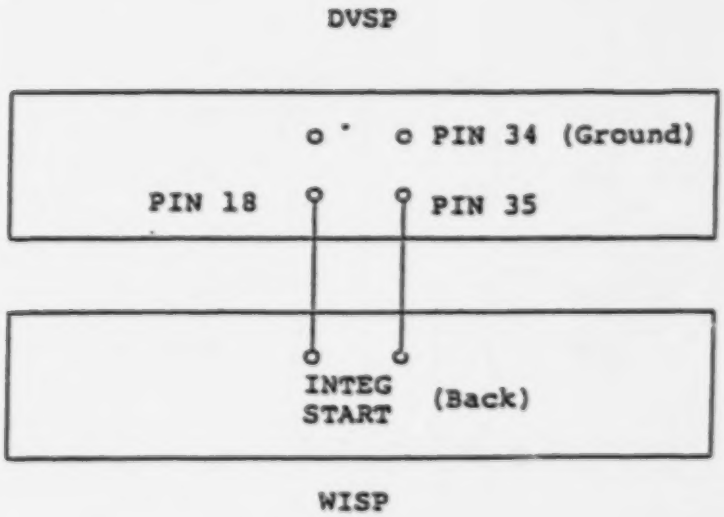


FIGURE 3.

Electrical Connections for Six Port Valve and Digital Valve Sequence Programmer

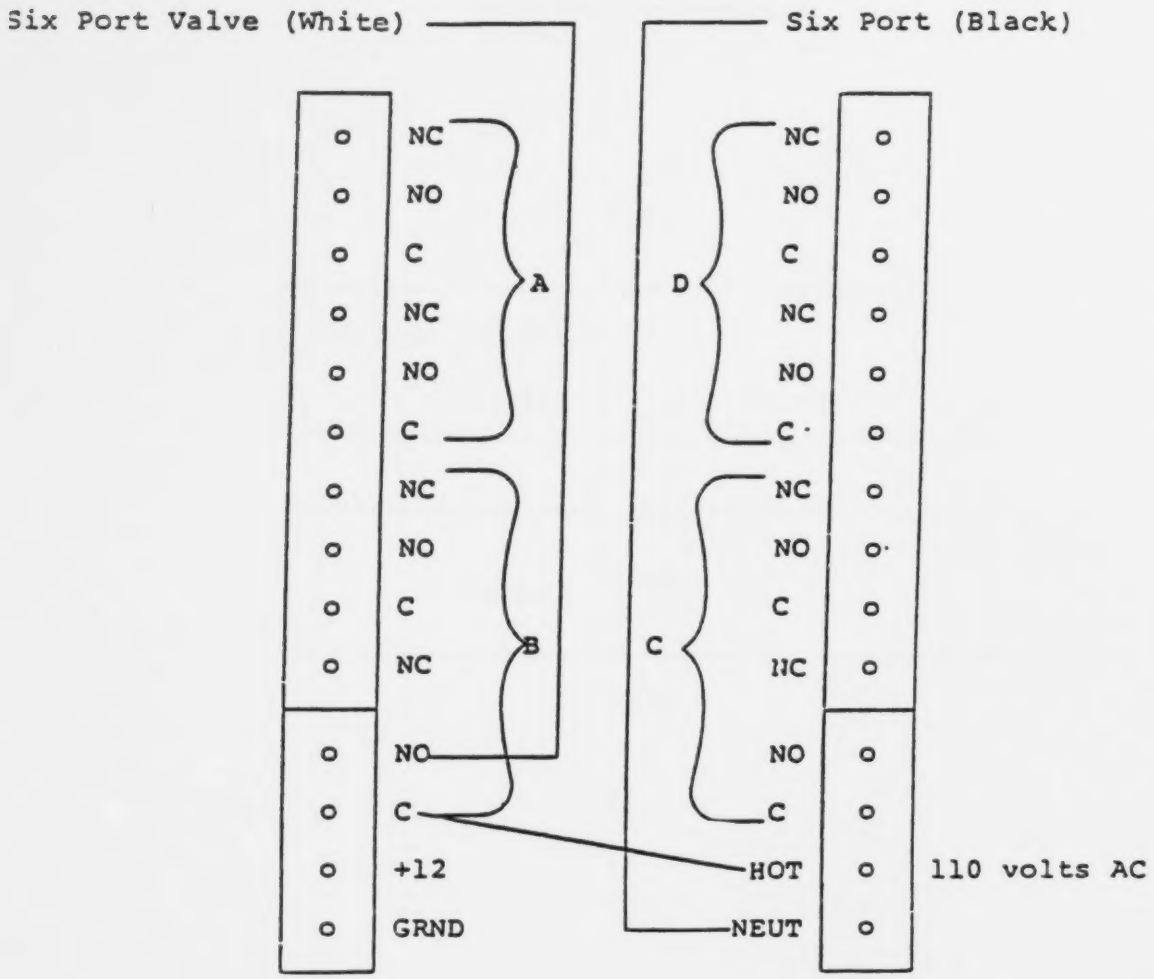


FIGURE 4.

The Electrical Connections between Spectrum and Colorimeter Recorder

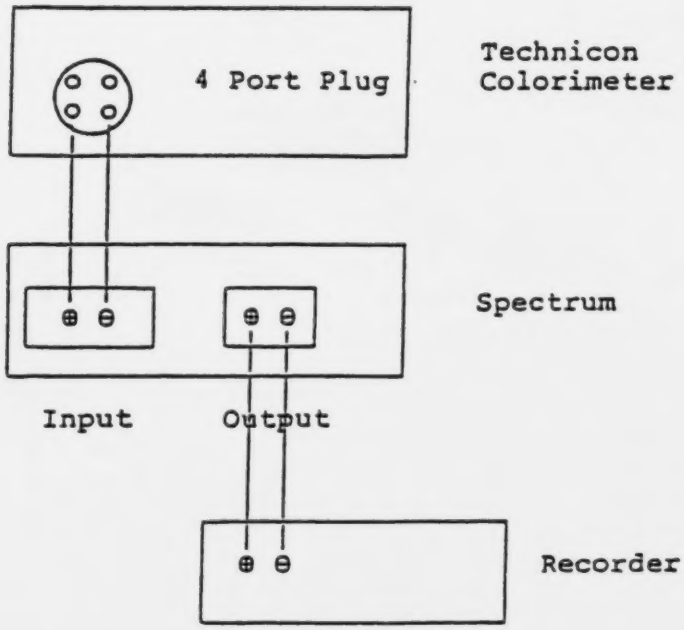
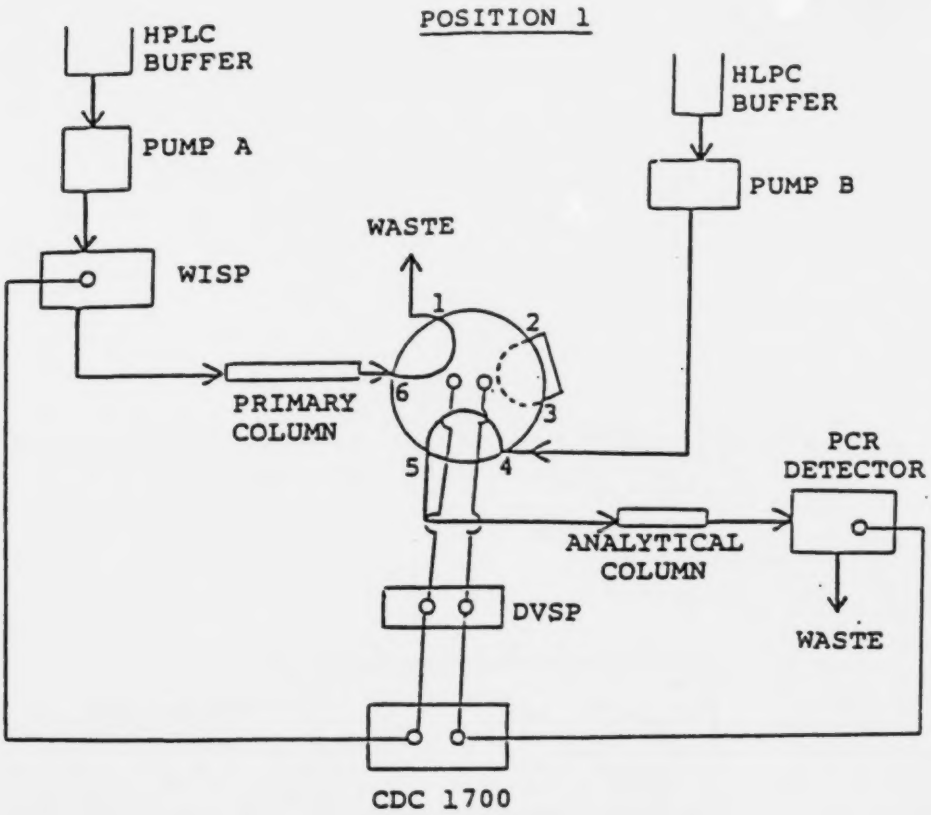
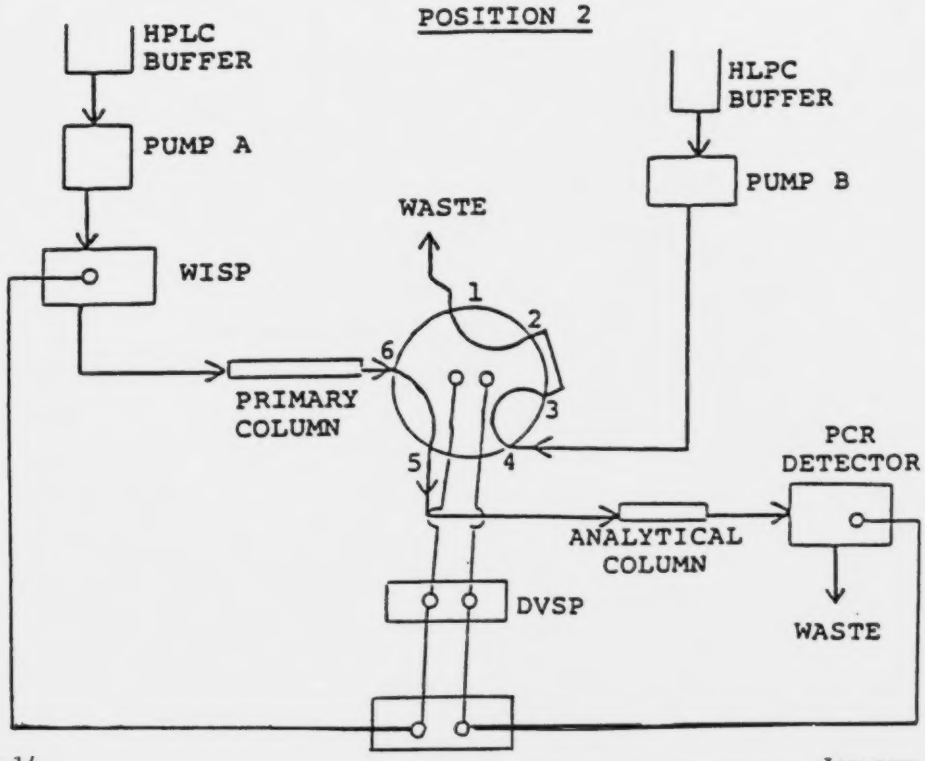


Figure 5

The Configuration of Column Switching Instrumentation





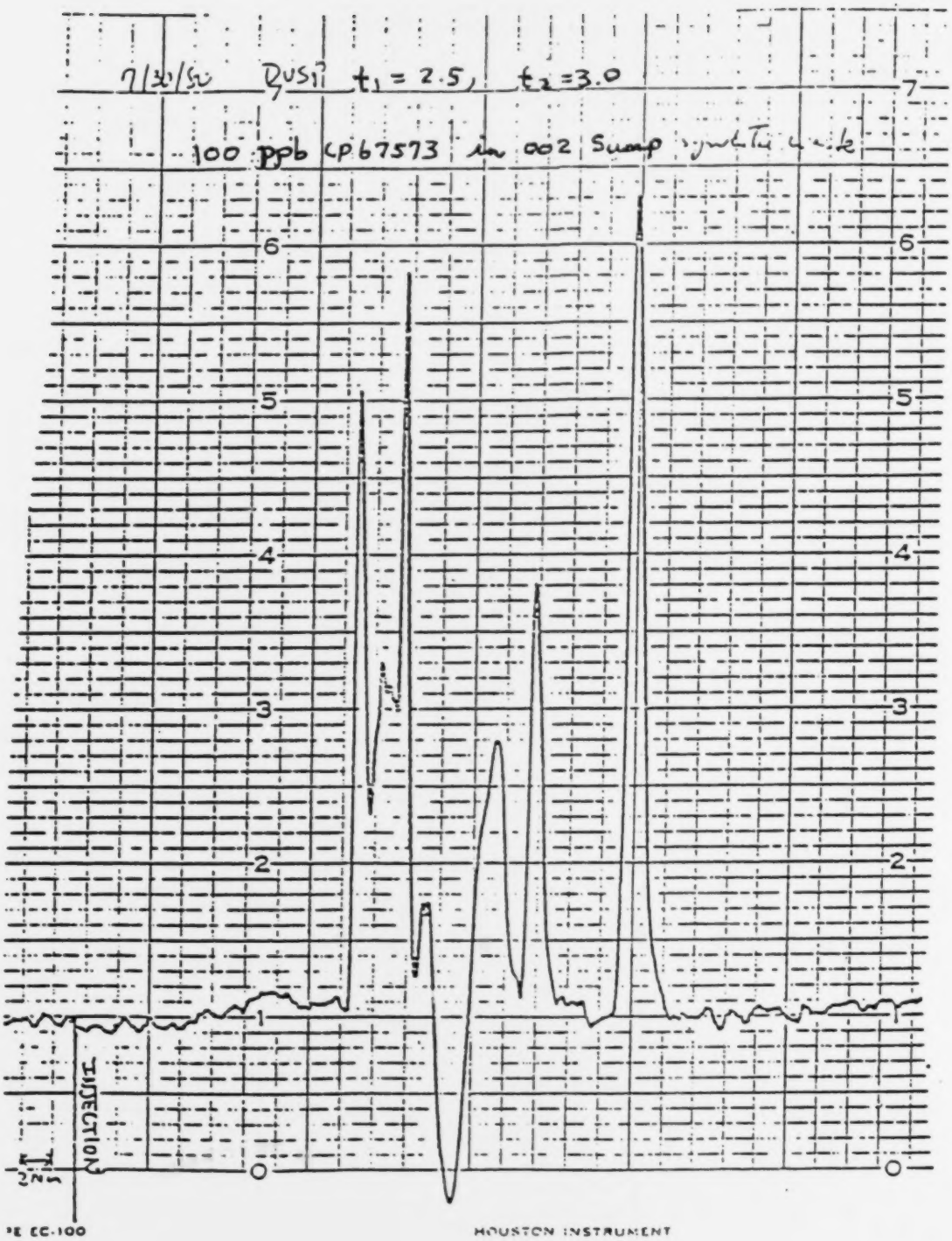


FIGURE 6.

Chromatogram of 100 ppb Glyphosate via
Column Switching Technique

Primary Column - 15cm x 4.0mm AG 50W-X8, 200-400 mesh, H^+ form

Analytical Column - 15cm x 4.6mm DuPont SAX

Mobile Phase (for both columns) -10g KH_4PO_4, 4% MeOH, pH 2.3 with H_3PO_4/41 H_2O

Post Column Reactor - Ninhydrin, Technicon Colorimeter, 570 nm

Injection Valve - 1ml by WISP

Flow Rate: 0.6 ml/min

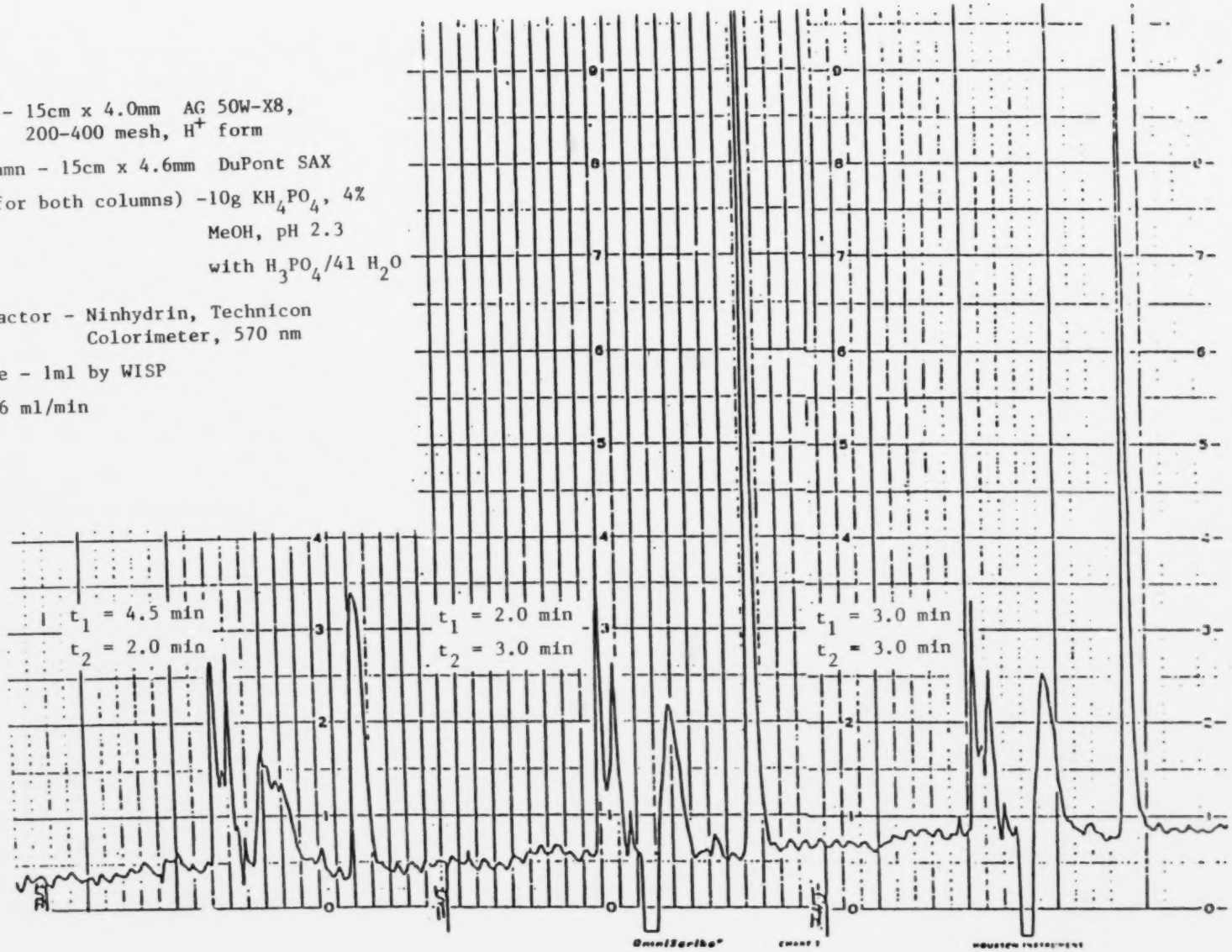


FIGURE 7.

Optimization of Peak Response by Adjusting Time Interval on DVSP.

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Water and Waste Management

TEST METHOD

DETERMINATION OF HEXAZINONE, TERBACIL, AND BROMACIL IN WASTEWATER

METHOD 141

1. Scope and Application

1.1 This method covers the determination of certain organonitrogen pesticides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Bromacil	82198	314-40-9
Hexazinone	—	51235-04-2
Terbacil	—	5902-51-2

1.2 This is a gas chromatographic-mass spectrometric (GC/MS) method applicable to the determination of the compounds listed above in effluent and waste streams.

1.3 The method detection limits (MDL) are 10 ug/L (bromacil) and 1 ug/L (hexazinone and terbacil). The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A measured volume of water sample (1000 mL) is extracted with methylene chloride. The extract is evaporated to 5 mL using a stream of nitrogen and 45°-50°C heat from a hot plate. Analysis is by GC/MS.

3. Interferences

Not Available.

4. Safety

4.1 All pesticides should be handled with care, avoiding any skin contact. Waste streams should be handled with care, avoiding any skin contact.

4.2 Although these pesticides are not considered to be highly toxic samples may irritate the eye, nose, throat, and skin:

. methoxychlor; LD_{50} in mice, 6000 mg/kg

. chloroneb; LD_{50} in rats, 11,000 mg/kg

. terbacil; LD_{50} in rats, 5000-7000 mg/kg

. hexazinone; LD_{50} in rats, 1690 mg/kg

. bromacil; LD_{50} in rats, 5200 mg/kg

4.3 Therefore, avoid personal contact and breathing of dust or contact with organic solutions. Perform all operations in a well ventilated area or hood, and clean up all spills promptly.

4.4 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Graduated cylinder, 100 mL

5.2.2 Separatory funnel, 2000-mL capacity, pear-shaped with Teflon stockcock.

5.2.3 Syringe, 10-uL capacity; Hamilton No. 801N., Supelco Inc., Bellafonte, PA 16823.

5.3 Hewlett-Packard Model 5992B -- gas chromatograph/mass spectrometer with flexible disc software. Hewlett-Packard, Avondale, PA 19311 (No substitute).

5.3.1 Column: 6-ft, 1/4-in O.D. x 2-mm I.D., glass-packed with 3% OV-101 on 80/100 mesh high performance Chromosorb W.

6. Reagents

6.1 Methylene Chloride -- Burdick & Jackson (distilled in glass).

7. Calibration

7.1 Establish GC/MS operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Analytical Stock Standard Solution (150 ug/mL)

7.2.1.1 Weigh 0.015 g (± 0.001 g) of the analytical standard methoxychlor, chloroneb, terbacil, hexazinone, and bromacil (Du Pont Bio-chemical Department) into separate 100-mL volumetric flask. Note the exact weight of each standard.

7.2.1.2 Dilute to volume with spectral quality methylene chloride.

7.2.1.3 Place the volumetric flasks in an ultrasonic bath for 5 minutes to complete dissolution.

7.2.1.4 Store at $4^{o}C$.

7.2.2 Analytical Working Standard Solutions -- Note: All working standard solutions must be prepared fresh daily.

7.2.2.1 Solution "A" (1.5 ug/mL):

Add the following volumes of each analytical stock solutions into a 100-mL volumetric flask: methoxychlor, 1000 uL; chloroneb, 1000 uL; terbacil, 1000 uL; hexazinone, 1000 uL; bromacil, 1000 uL. Dilute to volume with methylene chloride. Mix well.

7.2.2.2 Solution "B" (0.75 ug/mL):

Add the following volumes of each analytical stock solutions into a 100-mL volumetric flask: methoxychlor, 500 uL; chloroneb, 500 uL; hexazinone, 500 uL; bromacil, 500 uL. Dilute to volume with methylene chloride. Mix well.

7.2.2.3 Solution "C" (0.375 ug/mL):

Add the following volumes of each analytical stock solutions into a 100-mL volumetric flask: methoxychlor, 250 uL; chloroneb, 250 uL; terbacil, 250 uL; hexazinone, 250 uL; bromacil, 250 uL. Dilute to volume with methylene chloride. Mix well.

7.2.3 Inject 1.0 uL of standard solution "A" (run in duplicate).

7.2.4 Average the integrated area from the two runs and record.

7.2.5 Repeat steps 7.2.3 and 7.2.4 with standard solutions "B" and "C".

7.2.6 Prepare a standard calibration curve of area response vs. concentration.

7.2.7 Repeat steps 7.2.3 through 7.2.6 with each compound.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Transfer 1000 mL of wastewater sample to a 2000-mL separatory funnel via a 1000-mL graduate cylinder.

10.2 Extract with 75 mL of methylene chloride (carefully vent the separatory funnel).

10.3 Drain the methylene chloride layer (bottom) to a 250-mL beaker.

10.4 Repeat step 10.2 (twice) and combine the methylene chloride layer, for a total of three portions of methylene chloride.

10.5 Evaporate the methylene chloride using a stream of N_2 and 45°-$50^{\circ}C$ heat from a hot plate.

10.6 Concentrate to 5 mL and transfer to a 10-mL Kuderna-Danish (K-D) graduated receiver flask. Rinse with 5 mL methylene chloride and add to the K-D flask.

10.7 Evaporate to 1 mL using a stream of N_2.

11. Cleanup and Separation

Not Available.

12. GC/MS

12.1 Analyze the extract according to the conditions given in Table 1 for the desired compound. Run in duplicate.

12.2 Calibrate the system as described in Section 7.

12.3 Compare the SIM integrated area to the calibration curve to get the apparent compound concentration in mg/L (ppm).

13. Calibration

13.1 Calculate the concentration (mg/L) of each component using the following equation:

$$\text{mg/L (ppm)} \begin{matrix} \text{methoxychlor} \\ \text{chloroneb} \\ \text{terbacil} \\ \text{hexazinone} \\ \text{bromacil} \end{matrix} = \frac{\text{apparent concentration}}{1000}$$

13.2 Report the concentrations of methoxychlor, chloroneb, terbacil, hexazinone, and bromacil to two significant figures.

14. GC/MS Confirmation

14.1 GC/MS confirmation is included in the analysis method.

15. Method Performance

15.1 The 2s relative standard deviation of 50 ug/L standards from 10 analyses are shown on Table 2.

15.2 Typical detection limits are: terbacil, 1 ug/L; hexazinon, 1 ug/L; and bromacil, 10 ug/L.

16. References

16.1 "Determination of Methoxychlor, Chloroneb, Hexazinone, Terbacil, and Bromacil, Gas Chromatography/Mass Spectrometry (GC/MS) Method," Method No. W28.3032(R), September 25, 1980, E.I. duPont de Nemours Co., Inc., Wilmington, DE.

Table 1

Gas Chromatography of Organonitrogen Pesticides

Conditions for Terbacil

Flow (helium): 25 mL/min

Injection port temp.: 275°C

Column temp.: 220°C
Solvent time out: 1.4 min
Run time: 3.0 min

Electron Multiplier volts: 2800 volts

Integration sensitivity: 0.01

Area threshold: 10
Dwell time: 150 milliseconds/mass
S I M window: 0.1 (AMU)
Ion Masses
1 - 161.0
2 - 160.0
2 - 117.0

Conditions for Hexazinone

Flow (helium): 25 mL/min

Injection port temp: 275°C

Column temp.: 275°C isothermal
Solvent time out: 0.8 min
Run time: 5.0 min

Electron Multiplier volts: 2800 volts

Integration sensitivity: 0.005

Area threshold: 1
Dwell time: 150 milliseconds/mass
S I M Window: 0.1 (AMU)
Ion Masses

1 - 171.0
2 - 83.0
3 - 128.0

Conditions for Bromacil

Flow (helium): 25 mL/min

Injection port temp.: 275°C

Column temp.: 230°C
Solvent time out: 0.8 min
Run time: 5.0 min

Electron Multiplier volts: 2800 volts

Integration sensitivity: 0.005

Area threshold: 1
Dwell time: 150 milliseconds/mass
SIM window: 0.1 (AMU)
Ion Masses

1 - 205.0
2 - 207.0
3 - 162.0

Table 2

Relative Standard Deviation and Recovery

Pesticide	Relative Std. Dev., %	Recovery, %
terbacil	4.8	91
hexazinone	6.2	82
bromacil	3.1	87

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TEST METHOD

DETERMINATION OF ZIRAM IN WASTEWATER

METHOD 142

1. Scope and Application

1.1 This method covers the determination of ziram. The following parameter can be determined by this method:

Parameter	Storet No.	CAS No.
Ziram	—	137-30-4

1.2 This is a titration method applicable to the determination of the compound listed above in water.

2. Summary of Method

2.1 The sample is decomposed in an acid medium with CS_2 evolution. The evolved gas is passed through a lead acetate trap, removing H_2S and SO_2. The scrubber gas is collected in methanolic KOH, forming potassium Xanthate. The solution is neutralized to a phenolphthalein end point and then titrated with iodine solution.

3. Interferences

3.1 Large amounts of copper compounds

3.2 Calcium and sodium arsenates

3.3 Ethylene thiourea

3.4 H_2S, SO_2, and COS

4. Safety

4.1 Acetic acid vapors are irritating to the eyes and respiratory system. The liquid readily burns the skin. It is flammable and emits toxic fumes when heated to decomposition temperatures. Safety glasses and rubber gloves are mandatory when handling this chemical.

4.2 Dithiocarbamate samples may be hazardous.

4.3 Sulfuric Acid--when making dilute solutions, never add water to the acid, but always add (slowly) sulfuric acid to the water. This is a highly exothermic addition and care must be taken to avoid splattering due to heat. Sulfuric acid is very corrosive, causing severe burns to the skin. Contact with the eyes may result in total blindness. In case of eye contact, wash with copious quantities of water--SECONDS COUNT. After thoroughly washing the eyes (hold the lids apart), get immediate transportation to a hospital. Safety goggles and rubber gloves are mandatory when handling acid.

4.4 If heated too rapidly the sample may react forcibly with the sulfuric acid in the reaction flask.

4.5 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 See Figure 1

6. Reagents

6.1 30% acetic acid, aqueous

6.2 0.1N Iodine. For preparation and standardization, see Method No. 601.0 (reference procedure, not enclosd herein).

6.3 10% Lead acetate, aqueous

6.4 1% Phenolphthalein indicator, alcoholic, Reagent No. 606.0

6.5 2 N Potassium hydroxide, methanolic

6.6 Starch indicator, stabilized or laboratory-prepared, Reagent No. 607.0.

6.7 25% Sulfuric acid, Reagent No. 608.0

7. Calibration

Not Available.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Fart D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Refer to Figure 1. Fill the first two scrubbers about 2/3 full with lead acetate solution and insert glass-wool plugs in the towers to break up the bubble streams. Add (graduate) 100 mL of menthanolic potassium hydroxide to the last two scrubbing towers. Make sure that the towers are dry before adding the KOH. Attach a vacuum to the fourth tower.

10.2 Weigh (to the nearest mg) an approximately 0.1-g sample and transfer to a 250-mL round-bottomed reaction flask. The sample size must be increased for lower concentrations. Add several glass beads to prevent bumping and attach the flask to the reaction train.

10.3 Turn on the water condensor, and slowly add the sulfuric acid solution into the reaction flask. Adjust the vacuum to give slow bubbling in the towers.

10.4 Heat the reaction flask with a gas burner as rapidly as possible. Watch the reaction closely for a few minutes, for when the acid is boiling vigorously problems with foaming usually occur. Control the reaction by removing the flame as necessary and adjusting the vacuum to prevent solution from backing up in the tower.

10.5 After about 10 minutes or so, when the solvent is boiling steadily in the reaction flask, adjust the vacuum to give the least bubbling to prevent solution back-up. Occasional checking only is required from this point.

10.6 Continue boiling for 1/2 to 1-1/2 hours or until the acid solution looks clear. Remove the heat, disconnect the reaction flask and the towers.

10.7 Rinse contents of the third and fourth scrubbing towers into separate 500-mL Erlenmeyer flasks using about a total of 250-mL demineralized water on each. Do not combine the two flasks.

11. Cleanup and Separation

Not Available.

12. Titration

12.1 Put a Teflon-coated stirring bar in the flasks, and place them on magnetic stirrers. Start the solutions swirling, and add 3 drops phenolphthalein indicator to each.

12.2 To each flask add acetic acid just until the red color disappears. Immediately titrate with standardized iodine solution; when near the end point, add 5 cc of starch indicator and titrate to a faint, but definite, color change.

12.3 Prepare a blank by pipetting a 100-mL KOH solution into a 500-mL Erlenmeyer flask, adding 250 mL water and neutralizing with acetic acid solution as in step 12.2. Add the starch indicator at this point and continue with step 12.2.

13. Calculations

13.1 Factors

Compound	Factor
Amobam 42	12.300
Amobam 48	
Amobam M	19.330
Amobam T	25.537
Butyl Ziram	23.705
DBTU	22.315
Diram	14.325
DMTU	13.800
Ethyl Ziram	18.005
Methyl Ziram	15.285
PTU	15.019
Rocure-10	33.260
RZ-100	33.260

13.2 $$\frac{(A-B)(C)(D)}{(E)} = \text{percent Dithiocarbamate}$$

where: A = sample titrant
B = blank titrant
C = normality of iodine
D = factor from step 13.1
E = weight of sample taken in step 10.2

14. Method Performance

Not Available.

15. References

15.1 FTM 36.2/11/80

15.2 USDA 765.1, 4/55

15.3 "Dithiocarbamate Assay by CS_2 Evolution," Analytical Method No. 105.0-R1, Revised 7/28/82, Fike Chemicals, Inc., Nitro, WV.

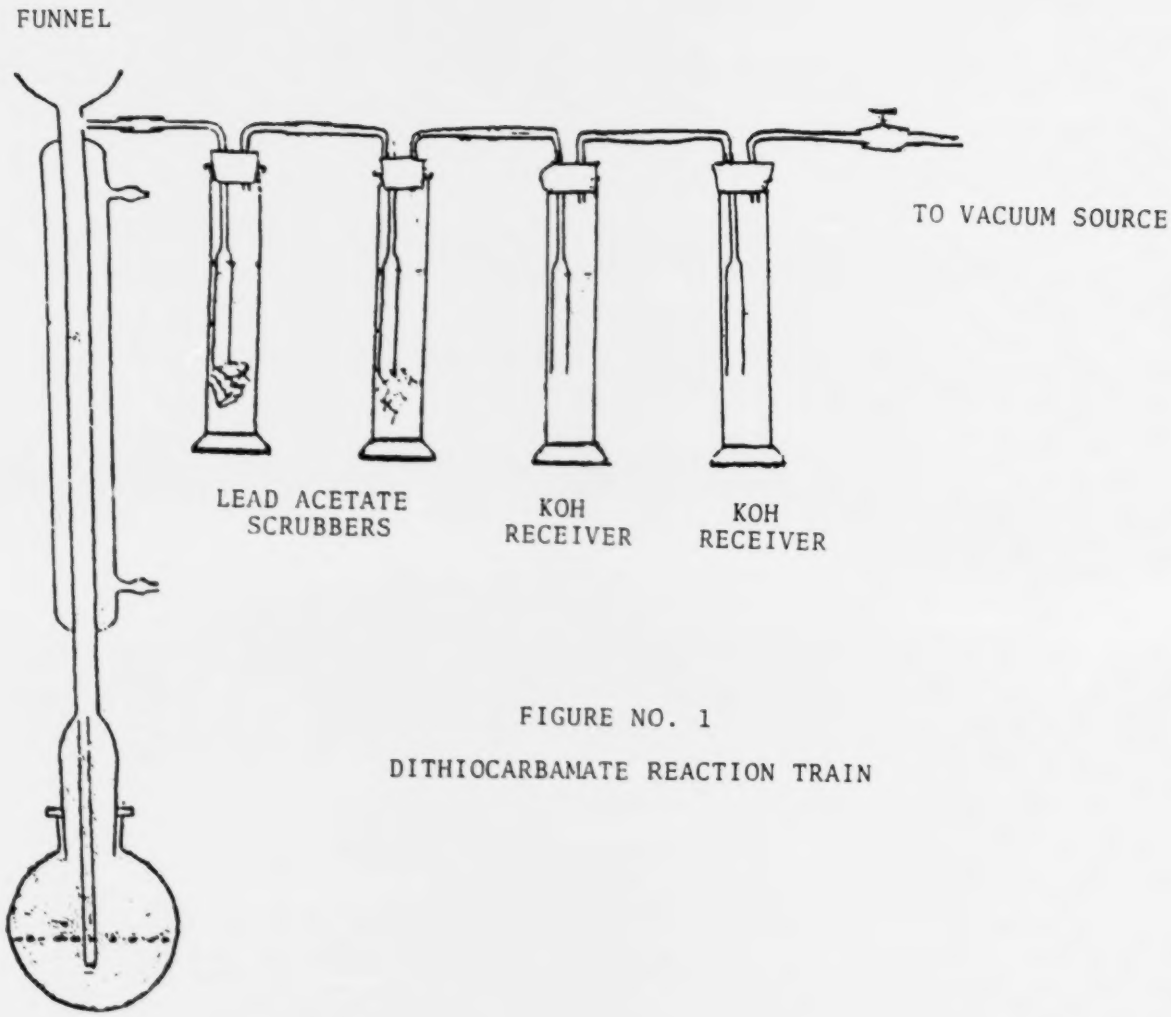

FIGURE NO. 1

DITHIOCARBAMATE REACTION TRAIN

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TEST METHOD

DETERMINATION OF PROPACHLOR IN WASTEWATER

METHOD 143

1. Scope and Application

1.1 This method covers the determination of propachlor (2-chlor-N-isopropylacetanilide).

Parameter	Storet No.	CAS No.
Propachlor	77729	1918-16-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in industrial streams at the mg/L (ppm) level and higher.

1.3 The sensitivity of this procedure is usually dependent upon the level of interferences rather than instrumental limitations.

2. Summary of Method

2.1 A measured volume of water sample (10 mL) is extracted with 10 mL of methylene chloride. The extract is analyzed without concentration using gas chromatography with a flame ionization detector.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis by running blanks.

3.2 Interferences coextracted from the sample will vary considerably from source to source, depending upon the diversity of the stream being sampled.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

5.1.1 Grab sample bottle--Amber glass washed and solvent-rinsed before use to minimize interferences.

5.1.2 Threaded screw-on bottle caps. Caps must be lined with Teflon.

5.1.3 Compositing equipment--Automatic or manual compositing system, incorporating glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated during sampling. No tygon or rubber tubing or fittings may be used in the system.

5.1.4 Clean 5-dram vial with Teflon-lined screw caps.

5.2 Glassware and Other Equipment

5.2.1 Gas Chromatograph--Analytical system complete with gas chromatograph with flame ionization detector, suitable for on-column injection and all column supplies, recorder, gases, and syringes. A data system for measuring peak areas is recommended.

6. Reagents

6.1 Methylene chloride, pesticide quality or equivalent

6.2 Propachlor standards

7. Calibration

7.1 Assemble the necessary gas chromatographic apparatus and establish operating parameters equivalent to those indicated in Table 1. By injecting calibration standards, establish the sensitivity limit of the detector and the linear range of the analytical system.

7.2 Calibration Procedure

7.2.1 Stock standards--Prepare stock standard solutions at a concentration of 1.00 ug/uL by dissolving 0.100 g of assayed reference material in pesticide-quality acetone or other appropriate solvent and diluting to volume in a 100-mL glass-stoppered volumetric flask. The stock solution is transferred to the glass-stoppered reagent bottles, stored in a refrigerator, and checked frequently for signs of degradation or evaporation, especially just prior to preparing working standards.

7.2.2 Prepare calibration standards that contain the compound of interest. The standards should be prepared at concentrations covering two or more orders of magnitude that will completely bracket the working range of the chromatographic system.

7.2.3 Calibrate the system daily with a minimum of three injections of calibration standards.

8. Quality Control

8.1 Before processing any samples, the analyst should demonstrate through the analysis of a distilled water blank that all glassware and reagents are interference free.

8.2 Standard quality assurance practices should be used with this procedure. Where doubt exists over the identification of a peak on the chromatograph, confirmatory techniques such as mass spectroscopy should be used.

8.3 Recoveries of spiked samples should be greater than 70% (actual recoveries averaged 90% for two samples).

8.4 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Grab samplers must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated from the time of collection until extraction.

9.3 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Using a 10-mL graduate, add 10 mL of sample to a 5-dram vial. Using the same graduate, add 10 mL of methylene chloride to the vial. Cap and shake either manually or mechanically for two minutes. After the phases separate, analyze aliquots from the lower (methylene chloride) layer.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended gas chromatographic column materials and operating conditions for the instrument.

12.2 Inject 2 to 5 uL of the sample extract. Smaller volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 uL and the resulting peak size.

12.3 If the peak size exceeds the linear range of the system, dilute the extract and reanalyze.

12.4 If the peak measurement is prevented by the presence of interferences, other techniques such as gas chromatography/mass spectrometry are required.

13. Calculations

13.1 Determine the concentrations of Propachlor according to the formula:

$$\text{Concentration (mg/L)} = (A)\ \frac{(B)}{(D)}\ \frac{(C)}{(E)}$$

where A = concentration of standard in ug/mL
B = sample peak response
C = total mL of extract
D = standard peak response
E = total mL of sample extracted

13.2 Report results in milligrams per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

14. Method Performance

Not Available.

15. References

15.1 "Propachlor (2-chloro-N-isopropylacetanilide),"Standard Method, Dow Chemical Co., Midland, MI.

Table 1

Chromatographic Conditions

Column Temperature	150°C isothermal
Carrier Gas	N_2 at 40 mL/min
Detector	Flame ionization at 220°C
Injection Port Temperature	210°C
Sample Size	2 Ul
Column:	1.8-m x 2-mm I.D. glass-packed with Permabond DEGS on Chromosorb WAW

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TEST METHOD

DETERMINATION OF FLUOMETURON IN WASTEWATERS

METHOD 144

1. Scope and Application

1.1 This method covers the determination of fluometuron.

Parameter	Storet No.	CAS No.
Fluometuron	—	2164-17-2

1.2 This is a thin-layer chromatographic (TLC) method applicable to the determination of the compound listed above in effluent streams.

1.3 The method detection limit (MDL) is 1 mg/L (ppm). A statement of "not detected" (ND) indicates only that the fluometuron is less than 1 mg/L (ppm).

2. Summary of Method

2.1 A measure volume of effluent sample (50 mL) is extracted with 10 mL of chloroform in a separatory funnel. The chloroform extract is isolated for spotting on pre-coated silica gel plates, which are then eluted with toluene/acetone (85/15, v,v). The plates are air dried to remove solvents, then exposed to chlorine gas in a saturated chamber for 45 seconds. The plates are removed, air dried, and sprayed with starch/KI solution.

3. Interferences

Not Available.

4. Safety

4.1 Plate exposure to chlorine and spraying with starch/KI are performed in a hood.

4.2 See EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Chambers: 23 cm x 28 cm x 7.5 cm from Analtech or equivalent.

5.2.2 Plate: Silica Gel G from Analtech 20 x 20 cm, 250-micron layer.

5.2.3 Capillary tubes, Kimax size 1.6-1.8 x 100 mm.

6. Reagents

6.1 Chloroform, reagent grade

6.2 Toluene

6.3 Acetone

6.4 Starch

6.5 Potassium Iodide (KI)

6.6 Distilled Water

6.7 Ethanol

6.8 Chlorine Gas

7. Calibration

7.1 Establish TLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 0.016 g $\pm$ 0.0005 g (to the nearest tenth milligram) of fluometuron standard into a 100-mL volumetric flask. Dilute this stock standard to the mark with $CHCl_3$. This stock solution is equivalent to 32 mg/L when compared to samples extracted 5:1. Dilutions of the stock solution are made such that additional standards are equivalent to 16, 8, 4, 2, and 1 mg/L when compared to samples extracted 5:1.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Extract 50 mL of the effluent sample with 10 mL $CHCl_3$ in a 125-mL separatory funnel. Isolate the $CHCl_3$ layer for spotting.

11. Cleanup and Separation

Not Available.

12. Thin Layer Chromatography

12.1 Table 1 summarizes the recommended operating conditions for TLC.

12.2 Calibrate the system as described in Section 7.

12.3 Eluent--One hundred milliter (100 mL) of eluent is prepared by mixing 85 mL of toluene and 15 mL of acetone by volume. The eluent should be prepared each day and allowed to equilibrate 1 hour before use. The eluent chamber is supersaturated and is equipped with a U-shaped filter paper lining.

12.4 Starch/KI Solution--To 1.5 g starch add 50 mL water. Heat and stir to the boiling point. To a second flask add 0.5 g potassium iodide plus 50 mL ethanol. Combine the starch, KI solutions, and ethanol into a TLC spray bottle.

12.5 One application of standards and samples is made with open-end capillary tubes (7 to 8 uL) onto a silica gel plate. The plate is placed in the chamber and the eluent allowed to migrate for 40 minutes to a distance of about 18 cm.

12.6 After elution, air dry plate to remove solvents. Then expose the plate to chlorine gas in a saturated chamber for 45 seconds. Remove plate and air dry 3 minutes or more. Spray the plate to produce a good contrast with starch/KI solution.

13. Calculations

13.1 Visual estimates of impurity concentrations in the sample are made based on spot size and intensity compared to the standards.

14. Method Performance

14.1 Detection Limit--A statement of "not detected" (ND) indicates only that the fluometuron is less than 1.0 ppm.

14.2 Relative Standard Deviation--The relative standard deviation of an analysis is approximately 30% relative to the actual concentration.

15. References

15.1 "TLC Determination of Fluometuron in Effluent Streams," ETM-95-1, June, 1982, Ciba-Geigy Corp., McIntosh, AL.

Table 1

Chromatographic Conditions

Eluent	Toluene/acetone, 85/15 by volume
Chamber	23 cm x 28 cm x 8.5 cm, supersaturated (filter paper lining)
Plate	Silica Gel G, Analtech 20 x 20 cm, 250-micron layer
Temperature	Ambient
Distance	18 cm
Time	40 min
Rf (fluometuron)	0.22
Rf (point of origin)	0.0

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TEST METHOD

DETERMINATION OF METRIBUZIN [SENCOR(TM)] IN WASTEWATER

METHOD 145

1. Scope and Application

1.1 This method covers the determination of metribuzin.

Parameter	Storet No.	CAS No.
Metribuzin [SENCOR(TM)]	39051	21087-64-9

1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in wastewater samples at concentrations of 1 mg/L or less.

2. Summary of Method

2.1 A measured volume of water sample (250 mL) is extracted with chloroform. The extract is partitioned with water, then evaporated to dryness. The residue is dissolved in methanol, filtered, and analyzed by HPLC with UV detector.

3. Interferences

3.1 All glassware used for analyzing wastewater samples must be pre-rinsed with chloroform and not previously used for any analysis other than wastewater. DO NOT USE PLASTIC WASH BOTTLES FOR ANY SOLVENTS.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Microsyringe, 50-uL with blunt-end needle (Adapted for use with a Valco valve)

5.2.2 Syringe, 1-mL

5.3 Water bath, 50° - $55^{o}C$

5.4 Filters, 13-mm diameter, Millipore Fluoropore #FHLP01300 or equivalent

5.5 Filter holder, Swinney, 13-mm stainless steel

5.6 Liquid chromatograph, Waters ALC 200 or equivalent, equipped with a uBondapak C_{18} column and a UV detector capable of measuring absorbances at 313 nm

6. Reagents

6.1 Acetonitrile, Burdick and Jackson "Distilled in Glass" or equivalent, degassed for 3 minutes under a vacuum in an ultrasonic bath

6.2 Chloroform, nanograde

6.3 Hydrochloric acid, 1N--Carefully add 21 mL of concentrated hydrochloric acid into a 250-mL glass-stoppered graduated cylinder containing approximately 200 mL of distilled water. Dilute to 250 mL with distilled water and mix thoroughly.

6.4 Methanol, ACS

6.5 Metribuzin (SENCOR) standard, technical grade (90-93% purity)

6.6 Sodium hydroxide, 1N--Carefully dissolve 10 g of sodium hydroxide pellets, ACS, in 250 mL of distilled water. Mix thoroughly.

6.7 Sodium sulfate, anhydrous, ACS

6.8 Water, deionized, filtered through activated carbon and a final 0.2-um filter and degassed for 3 minutes under vacuum in an ultrasonic bath

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 SENCOR standard solution, 0.10%--Weigh 0.11 - 0.12 + 0.0001 g of technical SENCOR (90 - 93% purity) into a 100-mL

volumetric flask. Dilute to volume with methanol, stopper, and mix thoroughly. This solution may be kept for one week only.

7.2.2 Pipet a 1-mL aliquot of the 0.10% SENCOR solution into a 10-mL volumetric flask, dilute to volume with methanol, and mix thoroughly. Label this solution 0.4 ppm SENCOR. (This is not the actual concentration, but is an equivalent value.) Prepare fresh daily.

7.2.3 Inject 10 uL of the 0.4-ppm SENCOR standard solution and continue to inject the standard until the peak heights are reproducible to within ± 5% of the previous injections. SENCOR has an approximate retention time of 35-42 minutes.

8. Quality Control

8.1 Follow EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample by shaking, then proceed immediately to step 10.2.

10.2 Measure 250 mL of the well-mixed sample into a 500-mL separatory funnel.

10.3 If the pH is above 7.0 or below 6.5, adjust it to 6.5 - 7.0 with 1N hydrochloric acid or 1N sodium hydroxide using pH indicator paper or a pH meter.

10.4 Extract three times by vigorously shaking for 1 minute each time with fresh 50-mL portions of nanograde chloroform. Collect the three chloroform extracts in another 250-mL separatory funnel. If an emulsion forms, centrifuge the emulsified layer and add the clear chloroform layer, obtained by centrifuging, to the second separatory funnel. Return the water layer to the first separatory funnel.

10.5 Add 50 mL of distilled water to the combined chloroform extracts in the second separatory funnel and shake for one minute.

10.6 Drain the chloroform layer into a 300-mL 24/40 boiling flask through a funnel containing 4 to 5 grams of anhydrous sodium sulfate retained on a small glass-wool plug (pre-rinsed with 10 mL of chloroform). Rinse the sodium sulfate three times with 10-mL portions of chloroform.

10.7 Place the flask on a rotoevaporator and strip off all of the chloroform, using a water bath at 50°C. Remove the flask and cool it to room temperature.

10.8 Remove any last traces of chloroform with a stream of dry air at room temperature.

10.9 Pipet 1 mL of methanol into the flask from step 10.8, stopper immediately, and rotate the flask so that the methanol washes the inside of the flask. DO NOT allow the methanol to get onto the neck or stopper.

10.10 Put a Fluoropore filter into a Swinney filter holder, then attach the filter holder to a 1-mL syringe. Remove the syringe plunger and hold the tip of the filter holder in the mouth of a 1-mL volumetric flask.

10.11 Using a disposable pipet, transfer the sample solution into the syringe barrel, replace the syringe plunger, and force the sample solution through the filter and into a 1-mL flask. Stopper the flask.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

12.2 Calibrate the instrument as described in Section 7.

12.3 Inject 10 uL of the sample solution from step 10.11 and immediately push the "Run" button on the programmer. Allow the sample to complete the entire program cycle to elute all of the nonpolar compounds.

12.4 If there is any doubt as to which peak in the sample scan is SENCOR, inject 7.5 uL of sample solution spiked with 2.5 uL of 0.4 ppm SENCOR standard solution. Compare the spiked scan with the scan from step 12.3 to identify the SENCOR peak in the sample scan.

12.5 If the SENCOR peak in the sample scan is greater than the standard scan (off scale), repeat steps 12.2 through 12.4 with a range setting of 0.1 on the Waters ALC 202 or at attenuation 4 on the P-E Series 3. NOTE: The standard should be about full scale.

12.6 Measure the peak height of the standard and sample to the nearest 0.1 mm; record as S and A, respectively.

13. Calculations

13.1 SENCOR, mg/L (ppm) = $\frac{0.4\ A}{S}$

14. <u>Method Performance</u>

Not Available.

15. <u>References</u>

15.1 "Determination of SENCOR[TM] in Waste Water by HPLC," Analytical Method TM B-34.62, Mobay Chemical Corp, Agricultural Chemical Div., Kansas City, MO.

Table 1

Chromatographic Conditions

Waters ALC 200

Range, AUFS	0.05
Injector loop, uL	10
Solvent A	Water, deionized (see Reagents)
Solvent B	Acetonitrile (see Reagents)
Flow rate, mL/min	2.0
Initial conditions (Solvent B, %)	13
Final conditions (Solvent B, %)	100
Time, minutes	50
Wavelength, nm	313
Curve	11

P-E Series 3

Attenuation	1
Flow, mL/min	2.0
%A	13.0
T_1, min	50.0
T1, %A	13.0
T1, curve	0
T2, min	0
Tpurge, min	10.0
Tpurge, flow, ml/Min	2
Tpurge, %A	99.9
Tequil, min	5
Tend	999
Time constant	1.0
Lamp	UV
Filter	0
Injector loop, uL	10
Solvent A	Acetonitrile (see Reagents)
Solvent B	Water, deionized (see Reagents)

PART B

CONTRACTOR ANALYTICAL METHODS

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TEST METHOD

DETERMINATION OF AOP, FERBAM, NIACIDE, ZAC, ZINEB, AND ZIRAM IN WASTEWATER

METHOD 401

1. Scope and Application

1.1 This method covers the determination of certain dithiocarbamate herbicides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
AOP	--	--
Ferbam	--	14484-64-1
Niacide	--	15339-36-3
ZAC	--	--
Zineb	--	12122-67-7
Ziram	--	137-30-4

1.2 This is a class determination. The total dithiocarbamates are expressed as ug/L as Ziram. Dithiocarbamates and thiuram disulfides have been shown to react along the pathway chosen for this determination.

1.3 This is a spectrophotometric method applicable to the determination of the compounds listed above in water and industrial waste.

1.4 The method detection limit (MDL) for these parameters is approximately 200 ug/L. The MDL for a specific wastewater may differ from this value, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 The sample is decomposed in a strong acid resulting in the evolution of CS_2. CS_2 is reacted with alcoholic potassium

hydroxide to form xanthates, which are measured spectrophotometrically at 380 nm.

3. Interferences

3.1 Only dithiocarbamates and thiuram disulfides have been shown to undergo the decomposition reaction to produce CS_2, but the analyst should be aware that any compound containing the S=C-S- moiety will undergo the decomposition reaction and thus produce interference.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Volumetric flasks, 10.0 mL, Class A, serialized

5.2.2 Capillary micro-pipets; 10-, 50-, and 100-uL sizes

5.2.3 Miscellaneous laboratory glassware

5.3 CS_2 generator (Figure 1 and 2), consisting of (in each set):

5.3.1 One 125-mL long-neck flask with ₮ 19/22 female neck joint

5.3.2 One 250-mL Erlenmeyer flask with ₮ 19/22 female neck joint

5.3.3 One 500-mL Erlenmeyer flask with ₮ 19/22 female neck joint

5.3.4 One j-stem addition funnel with ₮ 19/22 male joint

5.3.5 One condensor/funnel adapter with ₮ 15 side joint

5.3.6 Three M-receivers (micro) (Figure 2)

5.3.7 One hose adapter

5.4 Analytical balance -- Mettler H78AR or equivalent

5.5 HV-Vis spectrophotometer -- Beckman DU-2 is suitable, operated at 380 nm, using W source

5.5.1 Spectrophotometer cells, Pyrex with path length adjustment inserts to allow path lengths of 10 mm, 3 mm, and 1 mm

6. Reagents

6.1 Ziram Analytical Standard, U.S. EPA, Research Triangle Park, NC

6.2 Potassium hydroxide, analytical reagent grade

6.3 Methanol, 99.9%, spectrophotometric grade, Aldrich Chemical Company, Milwaukee, WI

6.4 Sulfuric Acid, analytical reagent grade

6.5 Water, distilled in glass, boiled prior to use

6.6 Solutions

6.6.1 0.5 KOH in methanol -- to 500 mL methanol, add 28 g KOH. Stir to effect solution. Make the solution up to 1 liter with more methanol. Mix thoroughly and store in a plastic vessel.

6.6.2 30% V/V H_2SO_4 -- to 600 mL water, slowly add 300 mL concentrated H_2SO_4. Mix well, and store, when cool, in a reagent bottle. Caution: Pour the acid into the water! Dilution of H_2SO_4 produces much heat. Use thick-walled glassware, and take suitable safety precautions.

7. Calibration

7.1 The sample is analyzed spectrophotometrically at 380 nm

7.2 Calibration Procedure

7.2.1 Make a solution of CS_2 in methanol to contain 0.1000 g CS_2/mL. This is the master stock solution. Due to the fugitive nature of carbon disulfide, this solution must be kept frozen when it is not in actual use, and it should be remade no less often than weekly. All dilutions must be remade daily.

7.2.2 Dilute 0.1 mL of the master stock solution with methanol in a 10-mL volumetric flask. Withdraw 0.1 mL for further dilution and freeze this solution immediately. Label the flask "C-dilution."

7.2.3 Dilute 0.1 mL of the first dilution to the mark in another 10-mL volumetric flask with methanol. Freeze this solution immediately. Label the flask "E-dilution."

7.2.4 Thaw the C-dilution. Pipet 1.0 mL of the solution into a culture tube containing 4.0 mL of 0.5N KOH in methanol.

7.2.4.1 Refreeze the dilution. Swirl the culture tube to mix the solutions.

7.2.5 After 5 minutes, determine the absorbance of the mixture at 380 nm in the spectrophotometer.

7.2.6 Using 4-cycle semi-logarithmic graph paper, plot weight of CS_2 (ordinate) versus absorbance (abscissa).

7.2.7 Repeat Steps 7.2.4 through 7.2.6 using the E-dilution.

7.2.8 Prepare dilutions using 0.2, 0.4, 0.6, and 0.8 mL of the master stock solution, as described in Step 7.2.1. Carry them through Steps 7.2.3 through 7.2.7.

8. Quality Control

8.1 The analyst should verify method recovery by fortifying samples with a dithiocarbamate standard in appropriate amounts. No less than 10% of the samples should be duplicate. An additional 5% of the samples should be rerun as recovery spikes. Each lot of reagents should be screened for interferences in the blank.

8.2 Standardization of the decomposition and verification of recovery process are accomplished by the following:

8.2.1 Accurately weigh 0.01000 g of technical Ziram into a polypropylene weighing boat.

8.2.2 Transfer the material quantitatively to the decomposition flask, using approximately 50 mL of zinc-free water.

8.2.3 Arm the apparatus with its dropping funnel and absorber bulb. Add 30 mL of 30% Aqueous H_2SO_4 to the dropping funnel before attaching the absorber bulb.

8.2.4 Start the magnetic stirrer and heat the flask.

8.2.5 Start the aspirator and open the needle valve slightly.

8.2.6 Add the acid to the flask.

8.2.7 Adjust the stopcock of the addition funnel and the aspirator needle valve until the bubbling in the receivers is gentle and steady.

8.2.8 Bring the liquid in the flask to a gentle boil.

8.2.9 Continue the boiling and aspiration for approximately 15 minutes. Use cooling water in the condensor to avoid distilling any of the acidified water.

8.2.10 When 15 minutes have elapsed, cool the flask, continuing aspiration until boiling ceases.

8.3 Remove the dropping funnel assembly from the apparatus, then stop the aspirator.

8.4 Dismount the receiver tube and combine its contents in a 10-mL volumetric flask.

8.4.1 Rinse the receiver tubes with methanol and transfer the rinsings to the volumetric flask.

8.4.2 Make the contents of the volumetric flask to the mark with methanol.

8.5 From the plot prepared in Step 7.2.6, determine the weight of CS_2 in the solution obtained in Step 8.4.2.

8.6 Calculate the efficiency of the decomposition from the standard as follows:

$$\frac{64 \times (\text{Weight of Ziram})(76/306)}{5} = \text{theoretical g of } CS_2/\text{mL} = A$$

$$(\text{weight of } CS_2 \text{ from step 8.2.1}) \div 5 = \text{actual g of } CS_2/\text{mL} = B$$

$$\text{Percent efficiency} = 100\ B/A$$

8.7 See reference listed in Step 15.1.

8.8 See EPA Quality Control procedure found in Part D of this document.

9. <u>Sample Collection, Preservation, and Handling</u>

9.1 See reference listed in Step 15.2.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. <u>Sample Preparation</u>

10.1 Assemble the CS_2 generation apparatus (Figure 1).

10.2 Attach the two micro-receivers in series, clamping the ball joints with the appropriate clamps.

10.3 Attach the aspiration apparatus to the last receiver.

10.4 Add approximately 2 mL of 0.5N KOH in methanol to each receiver.

10.5 Warm the stored sample to room temperature.

10.6 Agitate the sample thoroughly to effect resuspension of the heavy metal salts of dithiocarbamoic acids.

10.7 Using a 100-mL graduate cylinder, transfer 100 mL of the sample to a 250-mL decomposition flask.

10.8 Carry out the decomposition as described in Steps 8.2.3 through 8.4.2.

11. Cleanup and Separation

Not Available.

12. Analysis

12.1 Determine the absorbance of the sample at 380-nm wavelength, using 0.5N KOH in methanol as a reference.

12.2 Determine the weight of CS_2 in the sample from the standard curve prepared in Section 7.

13. Calculations

13.1 Calculate the weight of Ziram equivalent to the weight of CS_2 detected as follows:

$$\frac{[(\text{weight of } CS_2 \text{ evolved in ug})/72] \times 306}{\text{sample volume in liters}} = \text{dithiocarbamates ug/L as Ziram}$$

14. Method Performance

14.1 The method detection limit is 200 ug/L.

14.2 Table 1 presents precision and accuracy data for this method.

15. References

15.1 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewater prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.2 Environmental Science and Engineering, Inc. (ESE). 1979 Pesticide BAT Review Verification Sampling Protocol. Prepared for the U.S. EPA, Research Triangle Park, North Carolina.

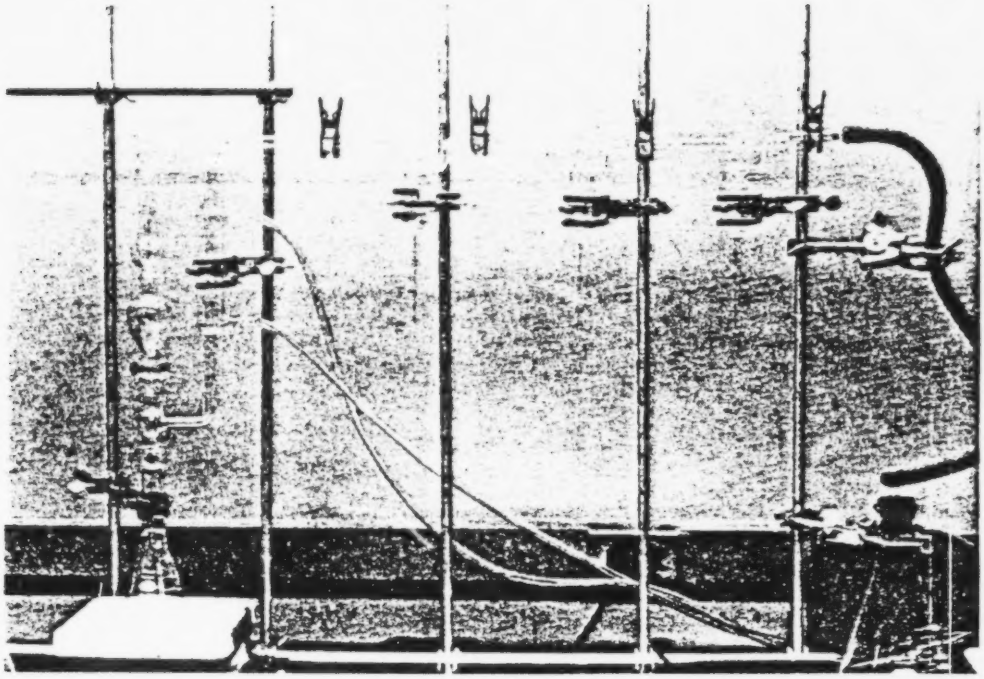
15.3 "Procedure for the Determination of Dithiocarbamates and Related Compounds," ESE Method 1200, February 16, 1979. Environmental Science and Engineering, Inc. Gainesville, FL.

Table 1

Precision/Accuracy Data

Parameter	Sample Type	mg/L Theoretical	mg/L Recovered	% Recovery
Ziram	DW	11.33	7.43	65.7
Ziram	DW	28.67	20.88	72.9
Ziram	WW	18.33	14.79	80.7
Ziram	WW	22.33	17.67	79.0

DW = Reagent Water
WW = Wastewater



FINAL ASSEMBLY OF ESE MODEL NO. 1 CS_2 EVOLUTION APPARATUS

Figure 1.

S & M CS_2 RECEIVER SHOWING FILLING LEVEL

Figure 2.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BENOMYL AND CARBENDAZIM IN WASTEWATER

METHOD 402

1. Scope and Application

1.1 This method covers the determination of benomyl and carbendazim. The following parameters can be determined by this method:

Parameter	Storet No.	CAS NO.
Benomyl	--	17804-35-2
Carbendazim	--	10605-21-7

1.2 This method will not separate carbendazim and benomyl from each other and, if both of these compounds are suspected to be present, the carbendazim results should be considered to be due to the sum of the contributions of each of these compounds.

1.3 The method detection limit (MDL) for carbendazim under the conditions used is 100 ug/L based upon a 5% response at 0.005 AUFS. The MDL for a specific wastewater may differ from those listed, depending upon the nature of the interferences in the sample matrix.

2. Summary of Method

2.1 Carbendazim is determined in wastewater by direct aqueous injection HPLC using a reverse-phase column. No cleanup or pretreatment was used.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Sample Filter: 5-mL Hamilton gas-tight syringe with Swinney Filter Apparatus and 5-u Nucleopore polyester filter.

5.2.2 High Pressure Liquid Chromatograph: Altex Model 322 or equivalent.

5.2.3 Injection valve: Rheodyne 7120 or equivalent

6. Reagents

6.1 HPLC Mobile Phase -- Dissolve 4.1 g of sodium acetate in 450 mL of HPLC-grade water. Add 50 mL glacial acetic acid. Mix thoroughly. Add 500 mL of HPLC grade methanol and mix thoroughly.

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

Not Available.

8. Quality Control

8.1 See reference listed in Step 15.1.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.2.

9.2 Follow EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Bring the sample to room temperature.

10.2. Thoroughly mix the sample to ensure homogeneity.

10.3 Prefilter the sample thorugh a 0.5-um polyester filter using a suitable syringe and Swinney Adapter.

10.4 High-level samples (greater that 500 ppm) should be diluted appropriately to the analytical working range (0.1 - 500 ppm) with the HPLC mobile phase.

10.5 Sample is now ready for injection.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

13. Calculations

Not Available.

14. Method Performance

14.1 The limit of detection for carbendazim under the conditions used is 100 ug/L based upon a 5% response at 0.0005 AUFS.

14.2 Accuracy/Precision data are presented in Table 2.

15. References

15.1 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters Prepared for the U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.2 Environmental Science and Engineering, Inc. (ESE). 1979. Pesticide Review Verification Sampling Protocol. Prepared for the U.S. EPA, Research Triangle Park, North Carolina.

15.3 "Determination of Carbendazim in Industrial Wastewater," ESE Method 1700, April 19, 1979, Environmental Science and Engineering, Inc., Gainesville, FL.

Table 1

Chromatographic Conditions

HPLC Conditions	
Column	25-cm x 4.6-mm I.D. packed with Zorbax ODS, 6-8 u
Pre-Column	5-cm x 2.1-mm I.D. packed with Whatman Co: Pell ODS
Flow Rate	1.0 mL/min
Injection Volume	50 uL
Temperature	Ambient
Detector	254-nm fixed wavelength U.V.
Mobile Phase	50-percent methanol/50-percent - 10-percent v/v glacial acetic acid, 0.10 M sodium acetate

Table 2

Precision/Accuracy Data

Parameter	Sample Type	ug/L Theoretical	ug/L Recovered	% Recovery
Carbendazim + Benomyl	WW	50	47.8	95.6
Carbendazim + Benomyl	WW	2.0	2.1	105
Carbendazim + Benomyl	WW	4.0	3.5	87.5
Carbendazim + Benomyl	WW	2.0	1.86	93

Parameter	Sample Type	ug/L Replicate 1	ug/L Replicate 2
Carbendazim + Benomyl	WW	2410	2430
Carbendazim + Benomyl	WW	8.16	8.24
Carbendazim + Benomyl	WW	2.66	2.66
Carbendazim + Benomyl	WW	0.89	0.91

WW = wastewater

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF CARBOFURAN IN WASTEWATER

METHOD 403

1. Scope and Application

1.1 This method covers the determination of carbofuran. The following parameter can be determined by this method:

Parameter	Storet No.	CAS NO.
Carbofuran	--	1563-66-2

1.2 This is a high-performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in water and industrial waste.

2. Summary of Method

2.1 Carbofuran and its metabolites (hydroxycarbofuran, keto-7-carbofuran, and carbofuran phenol) are determined by direct aqueous injection. Some samples require pre-concentration and all samples are filtered before determination by HPLC with UV detection.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Syringe, 100 mL gas tight, with Luer-Lok tip

5.2.2 Syringe, 5 mL gas tight, with Luer-Lok tip

5.2.3 Syringe, 100 uL, for filling the sampling loop.

5.3 Sample filtration kit, syringe adapter with 0.5-um polyester membrane filters, available from Altex, Inc., Berkeley, CA

5.4 Sep-Pak C_{18} Cartridges, Waters Associates, Milford, MA

5.5 High-Pressure Liquid Chromatograph -- Altex Model 322 or equivalent (Gradient Elution system is not necessary)

5.5.1 254-nm Ultraviolet Absorbance Detector with 8-uL flow cell, Altex Model 153 or equivalent

5.5.2 High pressure injection valve, capable of withstanding 5,000 psi, with 50 uL loop, Rheodyne 7 or equivalent

5.5.3 Zorbax ODS column, 4.6-mm I.D. x 25 cm

5.5.4 Guard column, 2.1-mm I.D. x 5 cm, packed with Whatman Co:pell-ODS. Obtained as Whatman "Column Survival Kit."

5.5.5 Potentiometric strip chart recorder, 1 mV and, 1- mV full ranges

6. Reagents

6.1 Water, Fisher HPLC Grade or equivalent

6.2 Methanol, Fisher HPLC Grade or equivalent

6.3 Glacial Acetic Acid, Analytical Reagent

6.4 Solutions

6.4.1 Acidic Water -- To 500 mL of HPLC-grade water in a 1-L volumetric flask, add 2.0 mL of glacial acetic acid. Dilute to the mark with HPLC-grade water and mix thoroughly.

6.4.2 HPLC Eluant -- (55% Methanol/44.8% Water/0.2% Acetic Acid)- To 300 mL of HPLC-grade methanol in a 1-L graduated cylinder, add 270 mL of acidified water and mix well. This

mixture may be degassed if bubbles occur in the HPLC detector.

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Prepare stock solutions of the various analyte standards in HPLC-grade methanol.

7.2.2 Prepare the appropriate dilute standards to bracket the concentration range of the samples to be analyzed by dilution of the stock solutions with the HPLC eluant.

7.2.3 Determine the average response factor for each analyte by measuring the peak height or peak area of the standard peaks in two standard solutions which bracket the sample concentration.

7.2.4 $\text{Response Factor} = \frac{\text{peak area or height}}{\text{concentration of standard}}$

8. Quality Control

8.1 See reference listed in Step 15.1.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.2.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Concentrations greater than 1 ug/mL

10.1.1 Bring the sample to room temperature.

10.1.2 Place a filter holder containing a 0.5-um membrane filter on the Luer-Lok of a 5-mL gas-tight syringe.

10.1.3 Remove the plunger and add 5 mL of the sample.

10.1.4 Replace the plunger, and force the sample through the filter.

10.1.5 Collect the filtrate in a small beaker. The sample is now ready for chromatography.

10.1.6 Note: Samples with concentrations of carbofuran and related compounds greater than 1000 ug/mL should be diluted (1:10) with the HPLC eluant before filtration in order to ensure the solubilities of the analytes.

10.2 Concentrations less than 1 ug/mL

10.2.1 Attach a Sep-Pak cartridge to a 5-mL Luer-Lok syringe.

10.2.2 Prepare the Sep-Pak cartridge for the sample as follows.

10.2.2.1 Force about 2.0 mL of HPLC-grade methanol through the cartridge.

10.2.2.2 Force about 10 mL of HPLC-grade water through the cartridge.

10.2.2.3 The cartridge is now prepared for the sample.

10.2.3 Measure 100 mL of sample into a 250 mL graduated cylinder.

10.2.4 Add 0.1 mL glacial acetic acid and mix well.

10.2.5 Place a prepared Sep-Pak cartridge on the Luer-Lok of a 100-mL gas-tight syringe.

10.2.6 Remove the plunger from the syringe and pour 100 mL of sample into the barrel.

10.2.7 Rinse the wall of the graduated cylinder with a few mL of HPLC-grade water and add the rinsing to the syringe barrel.

10.2.8 Replace the syringe plunger.

10.2.9 Force the liquid through the Sep-Pak cartridge, discarding the effluent.

10.2.10 Repeat steps 10.2.2 through 10.2.9 twice, for a total of 300 mL of sample.

10.2.11 Samples with high solids content may clog the cartridge after only 200 mL of sample has been processed. Such samples may either be pre-filtered or a concentration factor of 200:1 may be used.

10.2.12 Remove the cartridge from the 100-mL syringe and place it on the Luer-Lok of a 5-mL gas-tight syringe containing 2.0 mL of methanol.

10.2.13 Force the methanol through the cartridge.

10.2.14 Collect 1.0 mL of the eluate in a calibrated receiver (a Kuderna-Danish receiver or volumetric flask is adequate).

10.2.15 The sample is now ready for chromatography.

10.2.16 Determine carbofuran and its related compounds on high-pressure liquid chromatography using the conditions listed in Table 1.

11. Cleanup and Separation

11.1 Some degree of clean-up is provided by the Sep-Pak concentration procedure.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

12.2 Calibrate the system as described in Section 7.

13. Calculations

13.1 Concentration of analyte =

$$\frac{\text{peak area or height of analyte peak}}{\text{Average Response Factor}}$$

14. Method Performance

14.1 Organic-free water was spiked with several different concentrations of carbofuran and its metabolites and passed through the Sep-Pak Cartridges. Results are given in Table 2. The concentration factors were 300:1.

14.2 Precision and accuracy data for this method are given in Table 3.

15. References

15.1 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.2 Environmental Science and Engineering, Inc. (ESE). 1979. Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

15.3 "Procedure for the Determination of Carbofuran and Metabolites in Water and Industrial Wastes," ESE Method 1800, April 10, 1979, Environmental Science and Engineering, Inc., Gainesville, FL.

Table 1

Chromatographic Conditions

Pre-column	2.1-mm I.D. x 5-cm Whatman Co:Pell ODS
Column	4.6-mm I.D. x 25-cm Zorbax ODS
Mobile Phase	55% Methanol/44.8% Water/0.2% Acetic Acid, isocratic
Flow Rate	1.5 mL
Pressure	4300 psi
Injection Volume	50 uL
Detector	254 nm, 0.01 AUFS

Table 2

Recovery Study In Laboratory Water

Retention Time (min)	Compound	Conc. (ug/L)	% Recovery (Avg of 3 exp.)
3.1	Hydroxy-Carbofuran	7.5	69%
		75	59%
4.3	Keto-7-Carbofuran	3.9	83%
		38.6	99%
		580	99%
6.9	Carbofuran	8.1	83%
		81.2	99%
		1220	95%
8.3	Carbofuran Phenol	8.9	93%
		89	85%
		1330	78%

Table 3

Precision/Accuracy Data

Parameter	Sample Type	Theoretical (ug/L)	Acutal (ug/L)	% Recovery
Carbofuran	WW	11,400	11,400	100
Carbofuran	WW	114,000	111,000	97.3
Carbofuran	DW	417.6	429.2	102.7

Parameter	Sample Type	Replicate 1 (ug/L)	Replicate 2 (ug/L)
Carbofuran	WW	89	85
Carbofuran	WW	<25	<25
Carbofuran	WW	<25	<254
Carbofuran	WW	124,000	141,000

WW = wastewater
DW = reagent water

EPA

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Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF CHLOROBENZILATE, TERBUTRYN, AND PROFLURALIN IN WASTEWATER

METHOD 404

1. Scope and Application

1.1 This method covers the determination of certain triazines and other pesticides. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Ametryn	--	834-12-8
Chlorobenzilate	39460	510-15-6
Profluralin	--	26399-36-0
Terbutryn	--	886-50-0
Diazinon	39570	333-41-5
Atrazine	39033	1912-24-9

1.2 This is a gas chromatographic method (GC) applicable to the determination of the compounds listed above in wastewater.

2. Summary of Method

2.1 A 100-mL aliquot of sample is pH adjusted to pH 6.5 to 7.5 and extracted with hexane. The combined extract is dried and analyzed by gas chromatography with electron capture or thermionic detection.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnel, 2-liter

5.2.2 Kuderna-Danish apparatus

5.2.3 Micro-Snyder Column

5.3 Gas Chromatograph

5.3.1 Electron Capture Detector

5.3.2 Thermionic Detector

5.3.3 Column -- 1.8-m x 2-mm glass-packed with 3% STAP on 80/100 mesh Chromosorb WHP

6. Reagents

6.1 50% sodium hydroxide

6.2 50% sulfuric acid

6.3 Hexane, distilled-in-glass, Burdick and Jackson or equivalent

6.4 Anhydrous sodium sulfate

6.5 Dry nitrogen

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 The gas chromatograph is calibrated by the analysis of standard solutions in hexane of the compounds listed above. The range of standard concentrations should span the range of linear instrument response for each of the compounds.

8. Quality Control

8.1 The establishment and maintenance of quality control in the analyses of the environmental samples should include the determination of samples spiked with the compounds listed above, the determination of distilled water samples spiked with the compounds, and the determination of duplicate wastewater samples.

8.2 See reference listed in Step 15.2.

8.3 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.3.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Thoroughly mix the sample and transfer 100 mL of the wastewater sample to a 2-liter separatory funnel. Add 1400 mL of distilled, deionized water to the funnel and mix well. (The dilution of the sample prevents the formation of an emulsion during the extraction step.)

10.2 Adjust the pH of the diluted sample aliquot to 6.5 to 7.5 with 50% sodium hydroxide or 50% sulfuric acid.

10.3 In the separatory funnel extract the sample three times with 50 mL of ultrapure hexane. High pollutant concentrations may require use of a methylene chloride/hexane extracting solvent with a subsequent solvent transfer step to hexane prior to GC analysis.

10.4 Combine the three hexane extracts with hexane washings of the glassware. Remove traces of water from the mixture by passing it through a 75- to 100-mm by 12-mm O.D. column of anhydrous sodium sulfate. Dilute the dry hexane extract to 200 mL. Reserve 1-5 mL for analysis by gas chromatography.

10.5 Some samples may require additional concentration. Evaporate the remainder of the combined extracts to 5 to 10 mL in a 500-mL Kuderna-Danish (K-D) apparatus fitted with a 3-ball macro-Snyder column and a 10-mL calibrated receiver tube. Allow the K-D to cool to room temperature. Remove the receiver, add fresh boiling chips, attach a two-chamber macro-Snyder column and carefully evaporate to 1.0 mL (or when active distillation ceases). If the volume is greater than 1 mL, remove the micro-Snyder column and carefully evaporate to 1.0 mL with dry nitrogen.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

13. Calculations

Not Available.

14. Method Performance

14.1 Precision and recovery data are given in Table 3.

15. References

15.1 This procedure was adapted from the "Method for Triazine Pesticides in Industrial Effluents," in Federal Register, 38, No. 75, Part II.

15.2 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.3 Environmental Science and Engineering, Inc. (ESE). 1979. Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

15.4 "Analytical Procedures, Manufactured Pesticides and Related Compounds," Southern Research Institute Method, Southern Research Institute, Birmingham, AL.

Table 1

Chromatographic Conditions

Detectors:

(1) Electron capture detector (ECD), ^{63}Ni

(2) Thermionic detector (TD)

Operating conditions: ECD and TD

(1) Column temperature:

. Isothermal -- 190°C

. Program -- maintain at 90°C for 5 min, program from 90°C to 225°C at 10°/min, maintain at 225°C for 14 min

(2) Injection port temperature: 170°C

(3) Detector temperature: ECD-275C; TD-225°C

(4) Carrier gas flow rate: 45 mL/min ultra high purity nitrogen

(5) Auxiliary gas flow rates (or pressure settings): ECD -- 100 mL/min ultra high purity nitrogen purge gas

(6) Injection volume, 1 uL

Column: 1.8-m x 2-mm I.D. glass column packed with 3% STAP on 80/100 mesh Chromosorb WHP.

Table 2

Retention Times and Detection Limits

Column Temp	Compound	Retention Time, Min	Estimated Instrumental Detection Limit,* pg/uL ECD	TD
Isothermal	Profluralin	1.8	2	20
	Diazinon	2.5	40	1
	Atrazine	8.5	-	30
	Terbutryn	10.8	-	50
	Ametryn	12.2	-	90
	Chlorobenzilate	38.8	300	-
Program				
	Propazine	18.2	-	40
	Profluralin	15.1	10	100
	Diazinon	15.8	100	2
	Atrazine	19.3	-	40
	Terbutryn	20.1	-	40
	Ametryn	20.6	-	40
	Chlorobenzilate	27.6	200	-

* The concentration in hexane that is estimated to yield a peak height that is twice the background noise level in the absence of interferences.

Table 3

Precision/Recovery Data

Compound	Sample Type	Spike Level (ug/L)	% Recovery
Profluralin	WW	428	58.6
Terbutryn	WW	848	126
Chlorobenzilate	WW	83	104
Profluralin	WW	10,000	200
Terbutryn	WW	2,120	102
Chlorobenzilate	WW	2,070	149

Compounds	Sample Type	Level (ug/L)	RSD
Profluralin	WW	12,100	12.4%
Terbutryn	WW	144	5%
Chlorobenzilate	WW	488	8%
Profluralin	WW	92.4	13.5%
Terbutryn	WW	716	21%
Chlorobenzilate	WW	60.4	3%

WW = wastewater

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF 2,4-DB, 2,4-DB ISOBUTYL ESTER (2,4-DB IBE), AND 2,4-DB ISOOCTYL ESTER (2,4-DB IOE) IN WASTEWATER

METHOD 405

1. Scope and Application

1.1 This method covers the determination of certain phenoxy alkanoic acids and their esters. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
2,4-DB	--	94-82-6
2,4-DB IBE	--	51550-64-2
2,4-DB IOE	--	1320-15-6

1.2 This is a gas chromatographic (GC) method applicable to the compounds listed above in water and industrial effluents.

2. Summary of Method

2.1 An aliquot of sample is made basic with sodium carbonate and extracted with methylene chloride to isolate the phenoxy alkanoic esters. The sample is then acidified and extracted with methylene chloride to isolate the phenoxy alkanoic acids. The ester fraction is concentrated to a suitable small volume and analyzed by gas chromatography using electron capture or flame ionization detection. The acid fraction is evaporated to dryness, and the residue is methylated with diazomethane in ether. The excess diazomethane is evaporated. The methylated compound is analyzed by gas chromatography using electron capture or flame ionization detection.

3. Interferences

Not Available.

4. Safety

4.1 Diazomethane (CH_2N_2) is an extremely powerful methylating agent. It is toxic and highly explosive. It should be used only by experienced chemists in a well-equipped laboratory. It must not be generated or used in glassware with ground joints, or glassware which is etched or scratched, or has unpolished edges. Diazomethane should be generated and used only in a high-draft hood. Its solutions are moderately stable if no active protons are present and if they are kept in the dark at -20°C.

4.2 Excess diazomethane and its solutions can be destroyed by mixing with aqueous copper (II) sulfate and discarding the resulting solutions in a flammable waste can. Failure to exercise good laboratory techniques and observe proper precautions can lead to intoxication by exposure to the gas or its solutions, or to explosion of the generator or other glassware. (See Manufacturing Chemists' Association Handbook of Laboratory Safety.)

4.3 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnels, 1-liter, all Teflon

5.2.2 Filtering funnels, 60°C with long stems

5.2.3 Kuderna-Danish Concentrators, with 500-mL flasks, 10-mL receivers, and 3-ball Snyder columns

5.2.4 Pasteur pipets

5.2.5 2-mL septum-sealing vials, with Teflon-lined septum seals

5.2.6 Diazomethane generator, 0.1-mole size, Aldrich Chemical Co. Z10,025-0

5.2.7 Culture tubes with Teflon-lined caps, 15-mL size

5.2.8 Miscellaneous laboratory glassware

5.3 Glass wool, Pyrex

5.4 Funnel Racks

5.5 Analytical balance, Mettler H-78AR

5.6 500-mL heating mantle with variable transformer

5.7 Magnetic stirrer

5.8 High-draft hood, face velocity in excess of 50 cfm. This hood should be explosive-proof and provided with water and a drain, as well as 110-VAC electrical service.

5.9 Gas Chromatograph

5.9.1 Column -- 1.8-m x 2.0-mm glass packed with 15% OV-17 + 1.95% OV-210 on 100/120 mesh Chromosorb W-HP, or 1.8-m x 2.0-mm glass packed with 3% OV-101 on 100/120 mesh Chromosorb W-HP, or 1.8-m x 2.0-mm glass packed with a 3% OV-7 on 100/120 mesh Chromosorb W-HP. At least two of these columns should be used to allow confirmation of identification.

5.9.2 Electron Capture Detector

5.9.3 Flame Ionization Detector

6. Reagents

6.1 Methylene Chloride -- distilled in glass (Fisher "Pesticide Residue Grade" is acceptable)

6.2 Sulfuric Acid -- Analytical Reagent Grade

6.3 Sodium Carbonate -- Analytical Reagent Grade

6.4 Sodium Sulfate -- technical

6.4.1 Sodium sulfate, as received, is ignited at 600°C in a muffle furnace for 8 hours. It is cooled in a desiccator and stored in a glass reagent bottle.

6.5 Diazald (N-methyl, N-nitroso, p-toluenesulfonamide) Aldrich Chemical Co., D-2800-0

6.6 2- (2-ethoxy) ethanol, Aldrich Chemical Co., E-455-0

6.7 Diethyl Ether, absolute, Aldrich Chemical Co., 17296-4

6.8 Potassium Hydroxide, Analytical Reagent Grade

6.9 Standards of phenoxyalkanoic acids and esters

6.10 30% Sulfuric acid, aqueous -- 300 mL of concentrated sulfuric acid is added to 500 mL of demineralized water in a 2,000-mL heavy Pyrex beaker. When the solution has cooled somewhat, 200 mL of water is added. The mixture is mixed well and stored in a glass reagent jar when cool.

6.11 Ethereal Diazomethane. In a high-draft hood, assemble the diazomethane generator. Attach the condensor lines and start the flows of cooling water. If the temperature of the cooling water is judged to be too high, a cooling coil may be needed to provide the necessary efficiency. A temporary cooling coil can be made by coiling the 1/4-inch copper tubing around a small fire extinguisher, and connecting this coil upstream of the condensor. The coil should be immersed in an ice bath to provide cooling. Provide the reaction pot with magnetic stirring. Add 30 mL of 2 (2-ethoxyethoxy)-ethanol to the pot through the addition funnel. Begin heating the reaction pot. Start the magnetic stirrer. Add 10 mL of 50% aqueous KOH through the addition funnel. Add 30 mL of ether through the addition funnel. Weigh out 21.5 g (0.1 mol) Diazald. In a 250-mL beaker, add the Diazald to 150 mL of ether. Stir the solution well and place the majority of the solution in the addition funnel. When distillation of the ether begins, start addition of the ether solution of Diazald at a rate approximating the rate of distillation. Cool the receivers in an ice bath. A greenish-yellow hue in the distillate indicates evolution of diazomethane. Continue addition of Diazald until 2.5 g has been added. Rinse the beaker and addition funnel into the pot with small amounts of ether. Continue distillation until the distillate is water-white. When this occurs, cool the pot and rinse the contents into a waste down the drain. Combine the contents of the receivers and store, in amber glass bottles with Teflon-lined caps, in a freezer at -20°C until used or destroyed. Diazomethane solutions such as those generated by this method are stable under the storage conditions indicated for at least four weeks.

6.12 50% Potassium Hydroxide, aqueous -- add 10 g KOH to 10 mL of demineralized water. Stir the solution well and use immediately.

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 The identity of each compound should be determined by comparison of the adjusted retention times of the unknown and an authentic standard.

7.2.2 Quantitation should be accomplished from a regression line of at least five points.

7.2.3 Each compound should be reported without correction for recovery but with recovery stated. Acids should be reported as the free acid.

8. Quality Control

8.1 It is well known that reagents and glassware can contribute discrete artifacts which can interfere with determination.

Glassware and reagents must be shown to be free of interfering artifacts by the determination of a blank with each preparative "run."

8.2 Recovery of the analytes must be demonstrated in each sample matrix by fortifying the sample with appropriate concentrations of authentic standards. Not less than 5% of all samples should be fortified in this matter.

8.3 No less than 10% of all samples should be run in duplicate.

8.4 The esterification of the phenoxy alkanoic acids by diazomethane is quantitative. Low recoveries may result from evaporation of esters if the solvent is removed too rapidly or to dryness.

8.5 See reference listed in Step 15.1.

8.6 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.2.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Bring the samples to room temperature and agitate thoroughly.

10.2 Measure 500 mL of the sample into a 1-liter Teflon separatory funnel, using a graduated cylinder. Rinse the walls of the cylinder with methylene chloride, holding the rinsings temporarily in the cylinder.

10.3 Adjust the pH of the sample, in the funnel, to pH 10 or 11 using sodium carbonate.

10.4 When effervescence has ceased, add 100 mL of methylene chloride.

10.5 Cap the funnel and invert it. Vent the pressure to the hood.

10.6 Shake the funnel vigorously, taking care to vent the pressure to the hood periodically.

10.7 Allow the layers to separate. Drain the organic layer (lower) through sodium sulfate into a Kuderna-Danish concentrator.

10.8 Add another 100-mL portion of methylene chloride to the sample.

10.9 Repeat Steps 10.5 through 10.8 twice, so that the sample will have been extracted three times.

10.10 Combined extracts will contain phenoxyalkanoic esters. The

extracts are concentrated to 1.0 mL, transferred to septum capped glass vials, and stored at 4°C for later.

10.10.1 If electron-capture detection is to be used during chromatographic analysis, replace the solvent with iso-octane before storing the extract, using the following method.

10.10.2 Add 5 mL of ether to the sample in its Kuderna-Danish receiver.

10.10.3 Attach a micro-Snyder column and heat the apparatus in a hot water bath until the volume is reduced to about 0.5 mL.

10.10.4 Add another 5 mL of ether to the micro-concentrator and allow it to reflux until the volume is reduced to about 0.5 mL.

10.10.5 Add several drops of iso-octane to the concentrator and remove the last of the ether by heating the concentrator gently.

10.10.6 Make the sample to 1.0 mL and store it in a septum-sealing vial to await analysis. Label this extract "Ester Fraction."

10.10.7 If the ester fraction requires further cleanup, use modified Florisil Chromatography.

10.11 To the aqueous phase from Step 10.9, add 30% sulfuric acid (Step 6.11) until the pH of the sample is about 2.

10.12 Swirl the funnel until effervescence ceases.

10.13 Add 100 mL of methylene chloride to the sample. Cap the funnel and shake gently. Vent the pressure to the hood.

10.14 Agitate the funnel vigorously, venting the pressure to the hood.

10.15 Allow the layers to separate. Drain the organic layer (lower) directly into a Kuderna-Danish apparatus. Do not pass this extract over a drying agent, as low recoveries of the acids may result (through formation of the metal salts of the acids).

10.16 Repeat steps 10.13 through 10.15 twice.

10.17 Concentrate the combined acid extracts to about 2 mL in the Kuderna-Danish concentrator.

10.18 Transfer the concentrate quantitatively to a 15-mL culture tube. Transfer any crystals that have formed in the concentrate. Remove the last of the solvent by heating the sample in a water bath.

10.19 Add 5 to 8 mL of ethereal diazomethane. Cap the tube with a Teflon-lined cap and agitate it.

10.19.1 If the yellow color disappears, add more diazomethane.

10.19.2 Loosen the cap of the tube and gently heat it in a water bath to reduce the volume.

10.19.3 If the yellow color disappears, add more diazomethane.

10.19.4 When the color of the diazomethane remains for about 3 minutes, remove the residual diazomethane by heating the tube in a water bath. Add more ether if the volume is reduced too far (i.e., less than 0.5 mL). DO NOT allow the sample to go to dryness, as the analytes will be almost entirely lost.

10.20 Transfer the methylated sample to a 1-mL volumetric flask and dilute it to the mark with either methylene chloride or iso-octane. The choice of solvent will depend on the detector in use in the determinative step. Label this fraction "Acid Fraction."

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

12.2 Calibrate the system as described in Section 7.

13. Calculations

Not Available.

14. Method Performance

14.1 Precision and recovery data for this method are summarized in Table 2.

15. References

15.1 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.2 Environmental Science and Engineering, Inc. (ESE). 1979. Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

15.3 "Determination of Phenoxy Alkanoic Acids in Industrial Effluents," ESE Method 200, Environmental Science and Engineering, Inc., Gainesville, FL.

Table 1

Chromatographic Conditions

Electron-Capture detector (^{63}Ni) temperature	300°C
Flame Ionization detector temperature	300°C
Column Oven temperature	180°C isothermal
Injector temperature	200°C
Carrier gas (F.I.D.)	N_2, 30 mL/min
Carrier gas (E.C.D.)	Ar/5% CH_4, 30 mL/min
Hydrogen (F.I.D.)	30 mL/min
Air (F.I.D.)	240 to 300 mL/min, optimized for the detector

Table 2

Precision/Recovery Data

Compound	Sample Type	Spike Level (ug/L)	% Recovery
2,4-DB	WW	72,400	54
2,4-DB	WW	14,480	89

Compound	Sample Type	Replicate 1 (ug/L)	Replicate 2 (ug/L)
2,4-DB	WW	506,000	540,000
2,4-DB	WW	304,414	319,800

WW = wastewater

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF DINOSEB IN WASTEWATER

METHOD 406

1. Scope and Application

1.1 This method covers the determination of dinoseb.

Parameter	Storet No.	CAS No.
Dinoseb	—	88-85-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above in wastewater and effluents.

2. Summary of Method

2.1 A measured volume of water sample is adjusted to pH 6 and extracted with methylene chloride. The combined extracts are dried and concentrated to 10 mL. Analysis of the extracts is performed by gas chromatography using a flame ionization detector.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnel, 2 liter

5.2.2 K-D flask

5.3 Water Bath

5.4 Gas Chromatograph

5.4.1 6-ft x 2-mm glass column packed with 1.5% OV-17 + 1.95% QF-1 on Chromosorb W

5.4.2 FID Detector

6. Reagents

6.1 10N Sodium hydroxide

6.2 50% Sulfuric Acid

6.3 Methylene Chloride

6.4 Anhydrous Sodium Sulfate

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

Not Available.

8. Quality Control

8.1 See reference listed in Step 15.2.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.3.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Quantitatively transfer a 1000-mL aliquot into a 2-liter separatory funnel and adjust pH to 6 with 10N sodium hydroxide or 50% sulfuric acid.

10.2 Add 60-mL methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes. Allow the solvent to

separate from the sample, then draw the organic layer into a 500-mL flask.

10.3 Add a second 60-mL volume of solvent to the funnel and repeat the extraction procedure. Perform a third extraction in the same manner combining all the extracts.

10.4 Pre-dry with 1 to 2 g of sodium sulfate, then pass the organic extract through a chromatographic column containing 3 inches anhydrous sodium sulfate, and collect it in a 500-mL K-D flask equipped with a 10-mL ampul.

10.5 Concentrate the extract to approximately 5 mL in a K-D evaporator on a hot water bath.

10.6 Transfer the sample to a 10-mL volumetric flask and dilute to volume. Analyze the concentrate by gas chromatography.

11. Cleanup and Separation

Not Available.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph.

13. Calculations

Not Available.

14. Method Performance

14.1 The detection limit of this procedure is approximately 200 ug/L.

14.2 Table 2 gives recoveries of fortified samples.

15. References

15.1 "Methods for Benzidine, Chlorinated Organic Compounds, Pentachlorophenol and Pesticides in Water and Wastewater," EPA/EMSL, September 1978.

15.2 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.3 Environmental Science and Engineering (ESE). 1979. Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

15.4. "Procedure for Extraction and Analysis of Atrazine and Dinoseb at High Concentrations," TRW Method, TRW Systems Group.

Table 1

Chromatographic Conditions

Detector Temperature	190°C
Injector Temperature	190°C
Column Temperature	190°C
Carrier Gas	Helium
Flow Rate	30 mL/min

Column: 1% SP-1240DA on 100/120 Supelcoport (6-ft X 2-mm I.D. glass), although a 1.95% OV-17 + 1.5% QF-1 column packing is acceptable.

Table 2

Spike Recoveries

Compound	Sample Type	Spike Level (ug/L)	% Recovery
Dinoseb	WW	215	150
Dinoseb	WW	320	147

WW = wastewater

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF DINOSEB IN WASTEWATER

METHOD 407

1. Scope and Application

1.1 This method covers the determination of dinoseb. The following parameter can be determined by this method:

Parameter	Storet No.	CAS No.
Dinoseb	—	88-85-7

1.2 This is a gas chromatographic-mass spectrometric (GC-MS) method applicable to the determination of the compound above in wastewater.

1.3 The method detection limit (MDL) for dinoseb is approximately 200 ug/L.

2. Summary of Method

2.1 An aliquot of sample (pH adjusted to 2 or less) is extracted with methylene chloride. The combined extracts are dried and concentrated to 1 mL. An internal standard is added to the extract which is then analyzed by GC-MS.

3. Interferences

3.1 Emulsions -- The recovery of 85% of the added solvent will constitute a working definition of a broken emulsion. (You may correct the recovery of the first portion for water solubility of methylene chloride.)

3.2 Any of the following techniques that meet these criteria are acceptable.

3.2.1 Centrifugation of the emulsion layer after removal of any separated solvent.

3.2.2 Passage of the emulsion through a column plugged with a ball of methylene chloride-wet glass wool. The solvent used to wet the wool and to wash it after emulsion must be measured and subtracted from the total volume to determine 85% recovery.

3.2.3 Addition of excess extracting solvent sometimes breaks weak emulsions. Use excess solvent in the blank.

3.2.4 Let the emulsion stand for up to 24 hours.

3.2.5 Draw off the small amount of free solvent that separates and slowly drip it back in the top of the separatory funnel and through the sample and emulsion.

3.2.6 Stir with a glass rod, heating on a steam bath, add concentrated sodium sulfate solution, and sonicate.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Separatory funnels -- 2- and 4-L with Teflon stopcock

5.2.2 Continuous liquid-liquid extractors -- any such apparatus designed for use with solvents heavier than water and having a capacity of 2- to 5-L. Connecting joints and stopcocks must be of Teflon or glass with no lubrication.

5.3 GC/MS with conditions as described in Table 1

6. Reagents

6.1 6N Hydrochloric acid

6.2 Methylene chloride

7. Calibration

7.1 Establish GC/MS parameters equivalent to those indicated in Table 1.

7.2 External standardization is used to determine instrument response.

7.3 Inject varying dilutions of the standard mixure of acid/neutral compounds repeatedly into the GC/MS. Great care should be exercised to reproduce the injection volume. Prepare calibration curves of selected mass chromatogram peak areas as a function of the amount injected for each compound of interest. Use these calibration curves to determine the amount of each compound in the unknown wastewater sample extracts.

8. Quality Control

8.1 See reference listed in Step 15.2.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.3.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Sample Preparation

10.1.1 Blend the composite sample to provide a homogeneous mixture including a representative portion of the suspended solids that are present. Stirring with metal devices is acceptable for organic sampling.

10.1.2 Transfer the sample from the composite container through a glass funnel into a 2-L graduated cylinder and measure the volume. Then transfer to a 4-L separatory funnel or a continuous extractor. Rinse the cylinder with several portions of the first volume of extracting solvent. (Note: Either separatory funnel or continuous extraction is acceptable for isolation of the organics.)

10.2 Acid-Neutral Extraction

10.2.1 Separatory funnel extraction -- Adjust the pH of the sample with 6 N HCl to 2 or less. Use multirange pH paper for the measurement. Serially extract with 250 x 100- x 100-mL portions of distilled-in-glass methylene chloride. (About 40 mL of the first 250-mL portion will dissolve in the sample and not be recovered.) Shake each extract for at least 2 minutes.

10.2.2 Dry and filter the solvent extract by passing it through a short column of sodium sulfate. Concentrate the solvent by Kuderna-Danish (K-D) evaporation (distillation). The sodium sulfate should be prewashed in the column with methylene

chloride. (Note: Check sodium sulfate blank and, if necessary, heat in 500°C oven for 2 hours to remove interfering organics.) After drying the extract, rinse the sodium sulate with solvent and add to the extract.

10.2.3 Evaporate the extract to 5-10 mL in a 500-mL K-D apparatus fitted with a 3-ball macro-Snyder column and a 10-mL calibrated receiver tube. Allow the K-D to cool to room temperature. Remove the receiver, add fresh boiling chips, attach a two-chamber micro-Snyder column and carefully evaporate to 1.0 mL or when active distillation ceases. If required, remove the micro-Snyder column and carefully evaporate to 1.0 mL using an inert gas stream. Add the internal standard: 10 uL of 2 ug/uL d_{10}-anthracene (per each mL of extract). Mix thoroughly.

10.2.4 If it is to be overnight or longer before the extract is run by GC/MS, transfer it from the K-D ampul with a disposable pipet to a solvent tight container. The recommended container is a standard 2-mL serum vial with a Teflon Mininert valve. Store the extracts in the dark and at refrigerator or freezer temperatures.

10.3 Continuous Extractions -- If 85% solvent recovery cannot be achieved, start with a fresh aliquot of sample and extract by continuous extraction.

10.3.1 Adjust the pH of the sample as appropriate, pour into the extractor, and extract for 24 hours. When extracting a 2-L sample, using the suggested equipment, 2-L of blank water must be added to provide proper solvent recycle.

10.3.2 For operation, place 200-300 mL of solvent in the extractor before the sample is added and charge the distilling flask with 500 mL of solvent. At the end of the extraction remove the solvent from the distilling flask only, evaporate, and treat as described in the base-neutral extract section.

10.3.3 An analysis of a blank sample is required to evaluate the effect of the reagents and solvents used in the extraction and sample work up. All of the operations performed on the samples must be duplicated with the blank. Organic-free, distilled water is suitable for this blank sample.

11. Cleanup and Separation

Not Available.

12. GC/MS Analysis

12.1 Table 1 summarizes the recommended operating conditions for the GC/MS.

12.2 A computer system should be interfaced to the mass spectrometer to allow acquisition of continuous mass spectral scans for the duration of the chromatographic program. The computer system should also be equipped with mass storage devices for saving all data from GC-MS runs. There should be computer software available to allow searching any GC-MS run for specific ions and plotting the intensity of the ions with respect to time or scan number. The ability to integrate the area under any specific ion plot peak is essential for quantification.

12.3 Three conditions must be met to show the presence of a compound by GC/MS. First, the characteristic ions for the compound must be found to maximize in the same spectrum. Second, the time at which the chromatographic peak elutes must be within a specified window (usually $\pm$ 1 minute) for the retention time of the suspected component. Finally, the ratios of the three mass spectral peak heights must agree with the relative intensities of the standard spectrum of the suspected component with $\pm$ 20%.

12.4 For example, hexachlorobenzene elutes at 19.4 minutes. The characteristic ions are 284 (100%), 142 (30%), and 249 (24%). A plot of the intensities of these ions versus time or scan number in the 18.4 to 20.4-minute window is examined. If all three ions are present, the ratio of the peak areas is checked to verify that it is 100:30:24 $\pm$ 20 percent. If these three criteria are met, hexachlorobenzene has been identified in the sample.

13. Calculations

13.1 When a compound has been identified, the quantification of that compound will be based on the integrated area from the specific ion plot. Quantification will be performed by the internal standard method using deuterated anthracene. Response factors, therefore, must be calculated to compare the MS response for known quantities of each compound of interest with the internal standard. The Response Ratio (R) may be calculated as follows:

$$R = \frac{A_c}{A_a} \times \frac{C_a}{C_c}$$

where A_c is the integrated area of the characteristic ion from the specific ion plot for a known concentration, C_c. A_a and C_a are the corresponding values for the deuterated anthracene, internal standard. The relative Response Ratio should be known for at least two amounts that range over a factor of 10 (40 mg and 400 mg). Those compounds that do not respond at either of these levels may be run at concentrations appropriate to their response.

13.2 The concentration of the compound may now be calculated using:

$$C = \frac{A_c}{A_a} \times \frac{C_a}{R}$$

where C is the concentration of a component; A_c is the integrated area of the characteristic ion from the specific ion plot; R is the response ratio for this component; A_a is the integrated area of the characteristic ion in the specific ion plot, for deuterated anthracene; and C_a is the concentration of deuterated antracene in the injected contract.

14. Method Performance

14.1 The analytical recovery data presented in Table 2 were determined by spiking actual wastewater samples at levels approximately equal to those found in the neat samples or expected based on prior analysis. Percent recovery is calculated according to the formula

$$\text{Recovery, \%} = \frac{A - B}{C} \times 100\%$$

where:

A = Observed concentration (ug/L) in the spiked sample

B = Observed concentration (ug/L) in the neat sample

C = Concentration (ug/L) resulting only from the spike

14.2 Recovery for Dinoseb at 1060 ug/L was adequate and presented no problem. However, the GC/FID procedure has a detection limit at such a level that when sample dilution factors are included, no level below 200 ug/L can be accurately quantified. The spike level on the effluent sample was only 106 ug/L. These values obtained for the neat and spiked samples are not accurately known and subtracting these values from one another is meaningless.

15. References

15.1 "Sampling and Analysis Procedures for Screening of Industrial Effluents and Priority Pollutants," EPA-EMSL, April 1977.

15.2 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.3 Environmental Science and Engineering, Inc. (ESE). Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

15.4 "Method for GC/MS Analysis of Acid/Neutral Priority Pollutants," TRW Method, TRW Systems Group.

Table 1

Chromatographic Conditions

Packing	1% SP-1240DA on 100/120 mesh Supelcoport
Program	80°C -185°C at 8°C/min hold at 185°C for 15 min
Injector	190°C
Jet Separator	300°C
Carrier Gas	He at 30 mL/minute
Injection Size	1 uL

Column -- 6-ft x 2.0-mm I.D. glass

Table 2

Recovery Data

Compound	Sample Type	Spike Level (ug/L)	% Recovery
Dinoseb	WW	1060	91
Dinoseb	WW	106	Not Calculat See Ste

WW = wastewater

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF METHOMYL IN WASTEWATER

METHOD 408

1. Scope and Application

1.1 This method covers the determination of methomyl.

Parameter	Storet No.	CAS No.
Methomyl	39051	16752-77-5

1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in water.

1.3 The method detection limit (MDL) for methomyl in wastewater is approximately 1 ug/g; for "clean" water samples the MDL is approximately 0.01 ug/g. The MDL for a specific wastewater may differ from those given, depending upon the nature of interferences in the sample matrix.

2. Summary of Method

2.1 A measured volume of water sample (100 mL) is passed through two C_{18} Sep-Pak cartridges in series. The Sep-Paks are then eluted with acetonitrile, which is concentrated to 5 mL by a nitrogen stream. The extract is then analyzed by high performance liquid chromatography using an ultraviolet detector.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Syringe (10 mL)

5.2.2 Separatory funnel

5.3 Water Bath

5.4 Waters Associates Liquid Chromatograph

5.4.1 Model 6000-A pump

5.4.2 Model 440 UV detector

5.5 C-18 Sep-Pak Cartridge

6. Reagents

6.1 Acetonitrile, HPLC grade

6.2 Dry nitrogen

7. Calibration

7.1 Establish the HPLC conditions as described in Section 12.

7.2 Calibration Procedure

Not Available.

8. Quality Control

8.1 Two control samples (a blank" and a fortified spike at 10 ppm) are run with the samples.

8.2 See reference listed in Step 16.3.

8.3 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 16.4.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Clean the Sep-Pak cartridge by pumping 20 mL HPLC-grade acetonitrile through the cartridge using a 10-mL syringe.

10.2 Pump 10 mL distilled water through the cartridge using a 10-mL syringe.

10.3 Using apparatus set up as described in Figure 1, pour 100 g of the sample (10 g of the influent sample) into the separatory funnel. Open the stopcock and allow the water to drop into the long-stem funnel slowly enough that the funnel does not overflow. Use just enough vacuum to keep the water moving through the apparatus.

10.4 When all of the water has passed through the cartridges, disconnect the cartridges from the system.

10.5 Pump 10 mL HPLC-grade acetonitrile through the first cartridge into a 15 mL-centrifuge tube. Concentrate to <5 mL in a water bath using a stream of dry nitrogen. Repeat procedure with the second cartridge, eluting into the same centrifuge tube. Concentrate to 5 mL. Concentration is 100 g (10 g)/5 mL or 20 mg (2 mg)/uL.

10.6 Sample is ready for injection on LC.

11. Cleanup and Separation

Not Available.

12. Liquid Chromatography

12.1 Primary analysis was made using a Waters Associates Liquid Chromatograph equipped with a Model 6000-A pump and a Model 440 UV detector operated at a wavelength of 254 nanometers. A BONDAPAK C_{18} was used. The mobile phase was 40% CH_2CN/60% H_2O at 1.0 mL/min.

12.2 Residues were detected and measured as the "parent" methomyl compound; methomyl oxime (methyl-N-hydroxythioacetimidate) was also detected.

13. Calculations

Not Available.

14. GC/MS Confirmation

14.1 The residues were confirmed by GC-MS after conversion of the methomyl to methomyl oxime according to the procedure by Pease and Kirkland, (Journal of Agricultural and Food Chemistry, 16, 554-557, (1968) as modified in 1969.

14.2 Qualitative confirmation was also obtained by flame photometric detector.

15. Method Performance

15.1 The minimum detectable level for wastewater samples was approximately 1 ug/g (ppm); for "clean" water samples the MDL is approximately 0.01 ug/g .

15.2 Prior to running the samples, three controls were run to determine an approximate recovery for methomyl. Recoveries for the spiked controls were 41% for 1 ug/g and 66% for 10 ug/g. The "blank" control was equivalent to 0.02 ug/g (ppm).

15.3 Recovery of methomyl from a fortified sample spike at 10 ug/g was 70%.

15.4 Precision of this method is 41%.

16. References

16.1 "Journal of Agricultural and Food Chemistry", 16, 554-557 (1968), Pease and Kirkland.

16.2 The extraction procedure is based on a technique developed by Waters and Associates.

16.3 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

16.4 Environmental Science and Engineering, Inc. (ESE). 1979. Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

16.5 "Methomyl in Water," Pesticides Monitoring Laboratory Method, NASA/NSTL, Bldg. 1105, Bay St. Louis, MS 39529.

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TEST METHOD

DETERMINATION OF CYANAZINE IN WASTEWATER

METHOD 409

1. Scope and Application

1.1 This method covers the determination of certain triazine compounds. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Atrazine	39033	1912-24-9
Cyanazine	77780	21725-46-2
Simazine	39055	122-34-9
Propazine	39024	139-40-2

1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial wastewater.

1.3 The method detection limit (MDL) for the above listed parameters is 0.14 mg/L. The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matirx.

2. Summary of Method

2.1 An aliquot of sample is pH adjusted (to 7) and passed through a Sep-Pak C_{18} cartridge rinsed with methanol. The triazine compounds are trapped on the cartridge. The triazine compounds are eluted from the cartridge using an eluant of methanol/water (70%/30%). Analysis is by high performance liquid chromatography with an ultraviolet detector at 254 nm.

3. Interferences

Not Available.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

5.1.1 Syringes, 5-mL glass, gas-tight with Leur-lock tip

5.1.2 Syringe, 100-uL or greater for filling sample loop

5.2 Sep-Pak C_{18} cartridges, available from Waters Associates, Milford, MA

5.3 Altex Model 322 MP high-pressure liquid chromatograph with gradient capability or equivalent

5.3.1 Ultraviolet absorption detector -- 254-nm fixed waavelength with 8-uL flow cell, Altex model 153 or equivalent.

5.3.2 High pressure injection valve, capable of 5,000 psi, with 50-uL sample loop -- Rheodyne 7120 or equivalent.

5.3.3 Column: Zorbax ODS, C_{18}, 4.6-mm I.D. x 25-cm, available from DuPont, Wilmington, DE.

5.3.4 Guard column: 2.1-mm I.D. x 5-cm stainless steel column packed with Whatman Co:Pell ODS, available from Whatman, Clifton, NJ

6. Reagents

6.1 Methanol, distilled-in-glass, Burdick and Jackson or equivalent

6.2 Acetonitrile, UV-grade, distilled-in-glass, Burdick and Jackson or equivalent

6.3 Water, purified, or HPLC grade

6.4 10% v/v methanol/water solution. Prepare by adding 6 mL of methanol to 54 mL of water. Mix thoroughly.

6.5 70% v/v methanol/water solution. Prepare by adding 42 mL of methanol to 18 mL of water. Mix thoroughly.

7. Calibration

7.1 Establish HPLC parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Prepare stock solutions of the various analytes in methanol.

7.2.2 Prepare appropriate dilute standards to bracket the concentration range of the samples to be analyzed by dilution of the stock solutions with methanol.

7.2.3 Determine the linear range of the detector.

8. Quality Control

8.1 See reference listed in Step 15.1.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 See reference listed in Step 15.2.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 The sample is brought to room temperature.

10.2 The sample is thoroughly mixed to ensure uniformity.

10.3 Adjust pH of sample with 6N NaOH solution or 6N HCl solution to neutral pH-7. Check pH with pH paper.

10.4 Highly concentrated samples should be diluted to ensure complete sample dissolution and good recoveries through clean-up step.

10.5 Rinse the Sep-Pak C_{18} cartridge with 3 mL of methanol by attaching the long end of the cartridge to the Leur-lock end of a 5-mL gas-tight glass syringe and adding methanol to the barrel. Replace the plunger and pass the methanol through cartridge.

10.6 Rinse the cartridge with two 3-mL portions of organic-free water.

10.7 Remove the plunger from the barrel of a clean 5-mL gas-tight glass syringe and attach the long end of the cartridge to the Leur-lock tip.

10.8 Pipet 3.0 mL of sample into the syringe barrel.

10.9 Replace the syringe plunger carefully and invert syringe. Force trapped air out of syringe.

10.10 Slowly pass sample through cartridge with manual pressure at a

rate of approximately 1 mL/minute or alternatively attach syringe to syringe pump and pump at rate of 1 mL/minute.

10.11 Discard eluate. Carefully remove cartridge from syringe, so as not to lose the residual sample trapped on the cartridge, and place on clean surface.

10.12 Remove plunger from the syringe and replace the cartridge on the syringe tip.

10.13 Rinse the syringe barrel with approximately 1 mL of organic-free water and force water through cartridge by replacing plunger and employing manual pressure.

11. Cleanup and Separation

11.1 Pipet 1.0 mL of 10% v/v methanol/water into the syringe barrel of the syringe attached to the sample cartridge.

11.2 Replace the plunger and force the 10% v/v methanol/water solution through the cartridge.

11.3 Carefully remove cartridge from syringe and remove the plunger.

11.4 Replace the cartridge and pipet 3.0 mL of the 70% v/v methanol/water solution into the syringe barrel.

11.5 Replace syringe plunger, slowly pass the solution through the cartridge, and collect the eluate in a 10- or 25-mL Kuderna-Danish receiver.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph.

12.2 Calibrate the system as described in Section 7.

13. Calculations

13.1 Calculate the average response factor for each analyte by measuring the peak heights or peak areas of the standard peaks in two standard solutions which approximate and bracket the sample concentration.

13.2 $$\text{Response factor} = \frac{\text{peak area (or height)}}{\text{concentration of standard}}$$

13.3 $$\text{Concentration of analyte} = \frac{\text{peak area (or height) of analyte}}{\text{average response factor}}$$

14. Method Performance

14.1 Organic-free water was spiked with various concentrations of cyanazine, simazine, atrazine, and propazine. Three mL of this solution was passed through the clean-up procedure and analyzed by HPLC. Results are summarized in Table 2.

15. References

15.1 Jayanty, R.K. and Gutknecht, W.F. 1979. A Program of Quality Control/Quality Assurance for the Determination of Priority Pollutants in Pesticide Industry Wastewaters. Prepared for U.S. EPA, Process Measurements Branch, Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina. Research Triangle Institute, Research Triangle Park, North Carolina.

15.2 Environmental Science and Engineering, Inc. (ESE). 1979. Pesticide BAT Review Verification Sampling Protocol. Prepared for U.S. EPA, Research Triangle Park, North Carolina.

15.3 "Determination of Cyanazine, Simazine, Atrazine, and Propazine in Industrial Wastewater (Non-concentrative approach using Sep-Pak Cleanup)," ESE Method 710, Environmental Science and Engineering, Inc., Gainesville, FL.

Table 1

Chromatographic Conditions

Injection volume	50 uL
Column	Zorbax ODS, C_{18}, 4.6-mm I.D. x 25 cm
Flow Rate	1.0 mL/minute
Temperature	Ambient

Mobile phase:

A. To determine cyanazine and simazine only, isocratic, 40% acetonitrile/60% water.

B. To determine cyanazine, simazine, atrazine, and propazine

. Isocratic, 40% acetonitrile/60% water, for 19 minutes.

. To 70% acetonitrile/30% water in 5 minutes.

. Hold for 6 minutes.

C. The column should be flushed of extraneous peaks by changing mobile phase to 70% acetonitrile/30% water or 100% acetonitrile for a short period of time.

D. Sufficient and reproducible equilibration time should be employed before the next injection.

Table 2

Retention Times*

Parameter	Retention Time (min)
Cyanazine	9.6
Simazine	10.1
Atrazine	17
Propazine	27.8

* Under Condition B above

Table 3

Recovery Study in Laboratory Water

Compound	Spiked Concentration (mg/L)	Percent Recovery (Avg. of 2 exp)
Cyanazine	3.24	96.4
Simazine	3.26	97.1
Atrazine	3.04	97.0
Propazine	3.65	94.0

PART C

EMSL ANALYTICAL METHODS

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Water and Waste Management

TEST METHOD

DETERMINATION OF PENTACHLOROPHENOL SALT IN WASTEWATER

METHOD 604

1. Scope and Application

1.1 This method covers the determination of phenol and certain substituted phenols. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
4-Chloro-3-methylphenol	34452	59-50-7
2-Chlorophenol	34586	95-57-8
2,4-Dichlorophenol	34601	120-83-2
2,4-Dimethylphenol	34606	105-67-9
2,4-Dinitrophenol	34616	51-28-5
2-Methyl-4,6-dinitrophenol	34657	534-52-1
2-Nitrophenol	34591	88-75-5
4-Nitrophenol	34646	100-02-7
Pentachlorophenol	39032	87-86-5
Phenol	34694	108-95-2
2,4,6-Trichlorophenol	34621	88-06-2
Pentachlorophenol Salt	—	131-52-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for derivatization, cleanup and electron capture gas chromatography that can be used to confirm measurements made by flame ionization. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14.1) [1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix. The MDL listed in Table 1 for each parameter was achieved with a flame ionization detector. Comparable results were achieved when the derivatization cleanup and the electron capture detector were employed (See Table 2).

1.4 Any modification of this method, beyond that expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CRF 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the

ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A 1-liter sample of wastewater is acidified and extracted with methylene chloride using separatory funnel techniques. The extract is dried and concentrated to a volume of 10 mL or less. During the concentration step, the solvent is exchanged to 2-propanol. Flame ionization gas chromatographic conditions are described which allow for the measurement of the compounds in the extract [2].

2.2 A preliminary sample wash under basic conditions can be employed for samples having high general organic and organic base interferences.

2.3 The method also provides for the preparation of pentafluorobenzyl bromide (PFBB) derivatives for electron capture gas chromatography as an additional cleanup procedure to aid in the elimination of interferences [2,3].

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[4]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 12 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the method detection limits listed in Tables 1 and 2.

3.3 The basic sample wash (Section 10.2) may cause significantly reduced recovery of phenol and 2,4-dimethylphenol. The analyst must recognize that results obtained under these conditions are minimum concentrations.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

4.2 Special care should be taken in handling pentafluorobenzyl bromide, which is a lachrymator, and 18 crown 6 ether, which is highly toxic.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and capliner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - The sampler must incorporate glass containers for the collection of a minimum of 250 mL of sample. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2. Drying column - Chromatographic column 400-mm long x 19-mm ID with coarse frit.

5.2.3. Chromatographic column - 100-mm long x 10-mm ID, with Teflon stopcock.

5.2.4. Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.3 Vials - Amber glass, 10- to 15-mL capacity, with Teflon-lined screwcap.

5.4 Reaction flask - Pyrex glass, 15- to 25-mL round bottom flask with standard tapered joint, fitted with a water cooled condenser and U-shaped drying tube containing granular calcium chloride.

5.5 Boiling chips - Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.6 Water bath - Heated, with concentric ring cover, capable of

temperature control (± 2°C). The bath should be used in a hood.

5.7 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.8 Gas chromatograph - An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.8.1 Column for underivatized phenols - 1.8 m long x 2 mm ID glass, packed with 1% SP-1240 DA on Supelcoport (80/100 mesh) or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 11.1.

5.8.2 Column for derivatized phenols - 1.8 m long x 2 mm ID glass column packed with 5% OV-17 on Chromosorb W-AW-DMCS (80/100 mesh). The carrier gas is 5% methane/95% Argon at a flow rate of 30 mL/min. The column temperature is 200°C. This column has proven effective in the analysis of wastewaters for derivatization products of the parameters listed in the scope (Section 1.1), and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate columns are provided in Section 11.1.

5.8.3 Detectors - flame ionization and electron capture. The flame ionization is used when determining the parent phenols. The electron capture is used when determining the derivatized phenols. Guidelines for use of alternate detectors are provided in Section 11.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium hydroxide solution (10 N) - (ACS) Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium hydroxide solution (1 N) - (ACS) Dissolve 4g NaOH in reagent water and dilute to 100 mL.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

6.5 Sodium thiosulfate - (ACS) Granular.

6.6 Sulfuric acid solution (1+1) - (ACS) Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.7 Sulfuric acid (1 N) - (ACS) Slowly, add 29 mL H_2SO_4 (ACS, sp. gr. 1.84) to reagent water and dilute to one liter.

6.8 Potassium carbonate - (ACS) powdered.

6.9 Pentafluorobenzyl bromide (a-Bromopentafluorotoluene) - 97% minimum purity. NOTE: This chemical is a lachrymator. (See Section 4.2.)

6.10 18-crown-6 ether (1,4,7,10,13,16 - Hexaoxacyclooctadecane) - 98% minimum purity. NOTE: This chemical is highly toxic.

6.11 Derivatization reagent - Add one mL pentafluorobenzyl bromide and one gram 18 crown 6 ether to a 50-mL volumetric flask and dilute to volume with 2-propanol. Prepare fresh weekly. This operation should be carried out in a hood. Store 4°C and protect from light.

6.12 Acetone, hexane, methanol, methylene chloride, 2-propanol, hexane, toluene - Pesticide quality or equivalent.

6.13 Silica gel - Davison chemical, grade 923 (100/200 mesh) or equivalent. Activate at 130°C overnight and store in a desiccator.

6.14 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.14.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in pesticide quality 2-propanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be prepared at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.14.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268.

6.14.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 To calibrate the FIDGC for the analysis of underivatized phenols, establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure for FIDGC.

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 2-propanol. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure for FIDGC. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with 2-propanol. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard, (µg/L).

C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (< 10% RSD), the RF can be assumed to be nonvariant and the averge RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 To calibrate the ECGC for the analysis of phenol derivatives, establish gas chromatographic operating parameters equivalent to those indicated in Table 2.

7.4.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 2-propanol. One of the external standards should represent a concentration near but above the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.4.2 Each time samples are to be derivatived, simultaneously treat a one-mL aliquot of each calibration standard as described in Section 12.

7.4.3 After derivatization, inject 2 to 5 µL of each column eluate collected and tabulate peak height or area responses against the calculated equivalent mass of underivatized phenol injected. The results can be used to prepare a calibration curve for each compound.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyse a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in 2-propanol 1000 times more concentrated that the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 3, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X-R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3 s

Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[8] that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± S. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[8].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[9] should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Fill the sample bottle and at time of collection if residual chlorine is present, add 80 mg of sodium thiosulfate and mix well. U.S. Environmental methods 330.4 and 330.5 may be used for measurement of residual chlorine[10]. Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction[2].

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 For samples high inorganic content, the analyst may solvent wash the sample at a basic pH as prescribed in Section 10.2.1 and 10.2.2 to remove potential method interferences. Prolonged or exhaustive contact with solvent during the wash may result in low recovery of some of the phenols, notably phenol and 2,4-dimethyl phenol. For relatively clean samples, the wash should be omitted and the extraction, beginning with Section 10.3, should be followed.

10.2.1 Adjust the pH of the sample to 12.0 or greater with 10 N sodium hydroxide.

10.2.2 Add 60 mL of methylene chloride to the sample by shaking the funnel for one minute with periodic venting to release vapor pressure. Discard to solvent layer. The wash can be repeated up to two additional times if significant color is being removed.

10.3 Adjust the sample to a pH of 1 to 2 with sulfuric acid (1+1).

10.4 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and shake for two minutes. Allow the solvent to separate from the sample and collect the methylene chloride in a 250-mL Erlenmeyer flask. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation.

10.5 Add a second 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.6 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the KD if the requirements of Section 8.2 are met.

10.7 Pour the combined extract through a drying column containing three to four inches of anhydrous sodium sulfate, and collect in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL methylene chloride to complete the quantitative transfer.

10.8 Add one to two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will

not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling.

10.9 Increase the temperature of the hot water bath to 95 to 100°C. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of 2-propanol. A 5-mL syringe is recommended for this operation. Attach a micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of 2-propanol to the top. Place the micro-K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reached 2.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Add an additional 2 mL of 2-propanol through the top of the micro-Snyder column and resume concentrating as before. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum amount of 2-propanol. Adjust the extract volume to 1.0 mL. Stopper the concentrator tube and store in refrigerator at 4°C, if further processing will not be performed immediately. If the sample extract requires no further cleanup, proceed with flame ionization gas chromatographic analysis (Section 11). If the sample requires further cleanup, proceed to Section 12. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap vials.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Gas Chromatography - Flame Ionization Detector

11.1 Table 1 summarizes the recommended gas chromatographic column and operating conditions. This Table includes retention times and MDL obtained under these conditions. An example of the parameter separation achieved by this column is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

11.2 Calibrate the system daily as described in Section 7.1.

11.3 If the internal standard approach is used, the standard must be added to the sample extract and mixed thoroughly immediately before injection into the instrument.

11.4 Inject 2 to 5 µL of the sample extract using the solvent-flush technique[11]. Smaller (1.0 µL) volumes may be injected if automatic injectors are employed. Record the volume injected to the nearest 0.05 µL and the resulting responses in peak area or peak height units. If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

11.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound may be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.6 If the measurement of the peak response is prevented by the presence of interferences, an alternate gas chromatographic procedure is required. Section 12 describes a derivatization and column chromatographic procedure which has been tested and found to be a practical means of analyzing phenols in complex extracts.

12. Derivatization and Electron Capture Gas Chromatography

12.1 Pipet a 1.0-mL aliquot of the 2-propanol solution of standard or sample extract into a glass reaction vial. Add 1.0-mL of derivatizing reagent (Section 6.11). This is a sufficient amount of reagent to derivatize a solution whose total phenolic content does not exceed 0.3 mg/mL.

12.2 Add about 3 mg of potassium carbonate to the solution and shake gently.

12.3 Cap the mixture and heat it for four hours at 80°C in a hot water bath.

12.4 Remove the solution from the hot water bath and allow it to cool.

12.5 Add 10 mL of hexane to the reaction flask and shake vigorously for one minute. Add 3.0 mL of distilled, deionized water to the reaction flask and shake for two minutes. Decant a portion of the organic layer into a concentrator tube and cap with a glass stopper.

12.6 Pack a 10-mm ID chromatographic column with 4.0 grams of activated silica gel. After settling the silica gel by tapping the column, add about two grams of anhydrous sodium sulfate to the top.

12.7 Pre-elute the column with 6 mL of hexane. Discard the eluate and just prior to exposure of the sulfate layer to air, pipet onto the column 2.0 mL of the hexane solution (Section 12.5) that contains the derivatized sample or standard. Elute the column with 10.0 mL of hexane (Fraction 1) and discard this fraction. Elute the column, in order, with: 10.0 mL of 15% toluene in hexane (Fraction 2); 10.0 mL of 40% toluene in hexane (Fraction 3); 10.0 mL 75% toluene in hexane (Fraction 4); and 10.0 mL 15% 2-propanol in toluene (Fraction 5). All elution mixtures are prepared on a volume:volume basis. Elution patterns for the phenolic derivatives are shown in Table 2. Fractions may be combined as desired, depending upon the specific phenols of interest or level of interferences.

12.8 Analyze the fractions by electron capture gas chromatography. Table 2 summarizes the recommended gas chromatographic column and operating conditions. This Table includes retention times and MDL obtained under these conditions. An example of the parameter separation achieved by this column is shown in Figure 2.

12.9 Calibrate the system daily with a minimum of three aliquots of calibration standards, containing each of the phenols of interest that are derivatized according to the procedure (See Section 7.4).

12.10 Inject 2 to 5 µL of the column fractions using the solvent-flush technique. Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size, in area units or height. If the peak response exceeds the

linear range of the system, dilute the extract and reanalyze.

13. Calculations

13.1 Calculate the concentration of individual compounds in the sample determined by the flame ionization procedure (without derivatization) as indicated below.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L \quad \frac{(A)\,(V_t)}{(V_i)\,(V_s)}$$

where:

- A = Amount of material injected, in nanograms.
- V_i = Volume of extract injected (μL).
- V_t = Volume of total extract (μL).
- V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

$$\text{Eq. 3. Concentration, } \mu g/L = \frac{(A_s)\,(I_s)}{(A_{is})(RF)(V_o)}$$

where:

- A_s = Response for the parameter to be measured.
- A_{is} = Response for the internal standard.
- I_s = Amount of internal standard added to each extract (μg).
- V_o = Volume of water extracted, in liters.

13.2 Calculate the concentration of individual compounds in the sample when determined by the derivatization and electron capture procedure according to Equation 4:

$$\text{Eq. 4. Concentration, } \mu g/L = \frac{(A)\,(B)\,(V_t)\,(10)}{(V_i)\,(V_s)\,(C)\,(D)}$$

where:

- A = Mass of underivatized phenol represented by area of peak in sample chromatogram, determined from calibration curve in Section 7.4.3, in nanograms.
- B = Total volume of 2-propanol extract after concentration in mL.
- V_i = Volume of eluate injected (μg/L).
- V_t = Total volume of column eluate (μg/L).
- V_s = Volume of water extracted (mL).
- C = Volume of hexane sample solution added to cleanup column, in mL.
- D = Volume of 2-propanol extract used for derivatization in mL.

13.3 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.4 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in 8.4, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[11]. The MDL concentrations listed in Tables 1 and 2 were obtained using reagent water[12]. Similar results were achieved using representative wastewaters.

14.2 In a single laboratory (I.T. Enviroscience, Inc.), using spiked reagent water and wastewater samples, the average recoveries presented in Table 3 were obtained using a flame ionization detector[12]. The standard deviation of the percent recovery is also included in Table 3.

14.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

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12. "Development of Detection Limits, EPA Method 604, Phenols." Special letter report for EPA contract 68-03-2625. Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268.
13. **"Methods for Organic Chemicals Analyses in Municipal and Industrial Wastewater," July 1982, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45668.**

Table 1. *Chromatographic Conditions and Method Detection Limit*

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)
2-Chlorophenol	1.70	0.31
2-Nitrophenol	2.00	0.45
Phenol	3.01	0.14
2,4-Dimethylphenol	4.03	0.32
2,4-Dichlorophenol	4.30	0.39
2,4,6-Trichlorophenol	6.05	0.64
4-Chloro-3-methylphenol	7.50	0.36
2,4-Dinitrophenol	10.00	13.0
2-Methyl-4,6-dinitrophenol	10.24	16.0
Pentachlorophenol	12.42	7.4
4-Nitrophenol	24.25	2.8

Column conditions: Supelcoport (80/100 mesh) coated with 1% SP-1240 DA in 1.8 m long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min flow rate. Column temperature was 80°C at injection, programmed immediately at 8°C/min to 150°C final temperature. Method detection limits were determined with a flame ionization detector.

Table 2. *Silica Gel Fractionation and Electron Capture Gas Chromatography of PFBB Derivatives*

Parent Compound	Recovery (%) by Fraction[a]					Electron Capture	
	1	2	3	4	5	Retention Time (min.)	Method Detection Limit (µg/L)
2-Chlorophenol	-	-	90	1	-	3.3	0.58
2-Nitrophenol	-	-	-	9	90	9.1	0.77
Phenol	-	-	90	10	-	1.8	2.2
2,4-Dimethylphenol	-	-	95	7	-	2.9	0.63
2,4-Dichlorophenol	-	-	95	1	-	5.8	0.68
2,4,6-Trichlorophenol	-	50	50	-	-	7.0	0.58
4-Chloro-2-methyphenol	-	-	84	14	-	4.8	1.8
Pentachlorophenol	-	75	20	-	-	28.8	0.59
4-Nitrophenol	-	-	-	1	90	14.0	0.70

[a]Eluting solvent compositions as given in Section 12.7.
Column conditions: Chromosorb W-AW-DMCS (80/100 mesh) coated with 5% OV-17 packed in a 1.8 m long x 2.0 mm ID glass column with 5% methane/95% argon carrier gas at a flow rate of 30 mL/min. Column temperature isothermal at 200°C.

Table 3. *Single Operator Accuracy and Precision*

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
4-Chloro-3-methylphenol	82	15.0	0.70 - 3.5	21	3
2-Chlorophenol	67	14.8	0.74 - 3.7	21	3
2,4-Dichlorophenol	74	11.4	1.03 - 5.2	21	3
2,4-Dimethylphenol	51	14.0	0.82 - 4.1	21	3
2,4-Dinitrophenol	74	16.5	28.7	14	2
2-Methyl-4,6-dinitrophenol	86	12.4	34.6	21	3
2-Nitrophenol	67	12.9	0.80 - 4.0	21	3
4-Nitrophenol	45	7.9	15.9	21	3
Pentachlorophenol	79	8.8	21.0	21	3
Phenol	41	8.4	0.76 - 3.8	21	3
2,4,6-Trichlorophenol	71	14.5	1.20 - 6.0	21	3

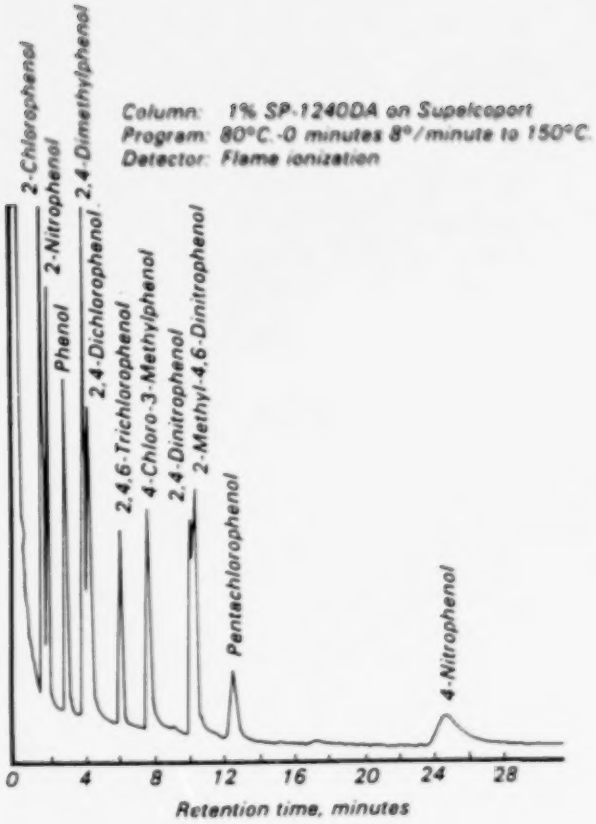

Figure 1. *Gas chromatogram of phenols*

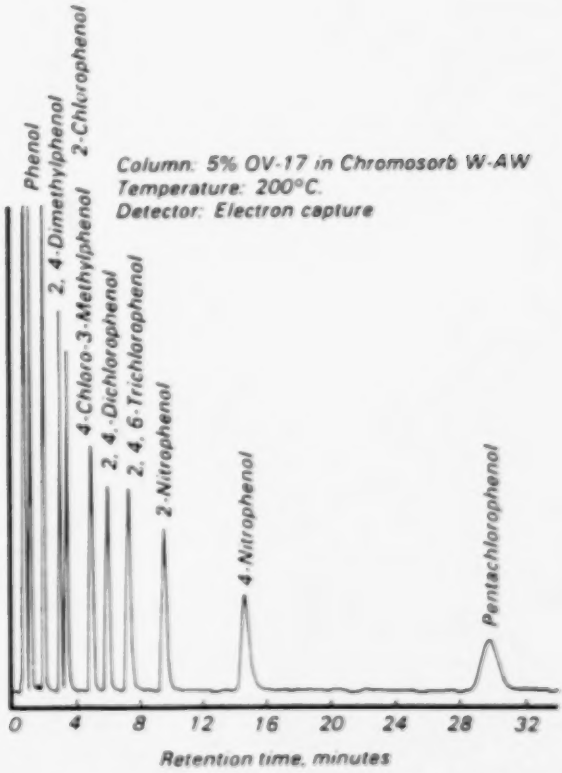

Figure 2. *Gas chromatogram of PFB derivatives of phenols.*

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF CHLOROBENZILATE, ETRIDIAZOLE, PROPACHLOR, DIBROMOCHLOROPROPANE (DBCP) IN WASTEWATER

METHOD 608.1

1. Scope and Application

1.1 This method covers the determination of certain organochlorine pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Chlorobenzilate	39460	510-15-6
Chloroneb	--	2675-77-6
Chloropropylate	--	5836-10-2
Dibromochloropropane	--	96-12-8
Etridiazole	--	2593-15-9
PCNB	--	82-68-8
Propachlor	--	1918-16-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The estimated method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 This method presents an extension in scope of Method 608. Further, the sample extraction and concentration steps in this method are essentially the same as several others in the 600-method series. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under Gas Chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column for chlorobenzilate and chloropropylate that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture (EC) gas chromatography.[1]

2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large late eluting peaks, especially in the 15 and 50% fractions from the Florisil column cleanup. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.[3,4] The interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mamalian carcinogens: chlorobenzilate; dibromochloropropane; and PCNB. Primary standards of these toxic compounds should be prepared in a hood.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with coarse fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with Ultrabond 20M (100/120 mesh) or equivalent.

5.6.3 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, isooctane, methylene chloride, methanol - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.5 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. Before use activate each batch at least 16 h at 130°C in a foil covered glass container.

6.6 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.6.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality isooctane and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.6.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.6.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value is suggested. This procedure[8] determines the adsorption from hexane solution of lauric acid, in mg, per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absense of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[9] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[9]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[10] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the four organochlorine pesticides listed in Table 3. It should also be applicable to the cleanup of extracts for PCNB.

11.2.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.2.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.2.3 Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane

(V/V) (Fraction 2), into a second K-D flask. Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3), into a separate K-D flask. The elution patterns for four of the pesticides are shown in Table 3.

11.2.4 Concentrate the eluates by standard K-D techniques (Section 10.6), substituting hexane for the glassware rinses and using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[11] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended.

A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[12]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[13]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[14] The MDL

concentrations listed in Table 1 were estimated from the response of an electron capture detector to each compound. The estimate is based upon the amount of material required to yield a signal five time the GC background noise, assuming a 5-µL injection from a 10-mL final extract of a 1-liter sample.

15.2 In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained after Florisil cleanup.[1] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND ESTIMATED METHOD DETECTION LIMITS

Parameter	Column 1 Temperature °C	Column 1 Retention Time (Min)	Column 2 Retention Time (Min)	Estimated MDL (µg/L)
Dibromochloropropane	100	3.1	--	0.04
Etridiazole	140	1.3	--	0.04
Chloroneb	150	2.0	--	0.04
Propachlor	150	3.8	--	1.0
PCNB	160	2.4	--	0.06
Chloropropylate	215	3.6	8.4	0.2
Chlorobenzilate	215	3.8	10.7	0.2

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperatures are listed above. An electron capture detector was used with this column to estimate the MDL.

Column 2 conditions: Ultrabond 20M (100/120 mesh) packed in a 1.8 m long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature is 200°C.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Sample Type	Background µg/L	Spike µg/L	Mean Recovery (%)	Standard Deviation (%)	Number of Replicates
Chlorobenzilate	MW	ND	10.5	74	7.2	6
	MW	ND	52.5	97	3.2	7
Chloroneb	MW	ND	18.1	92	2.9	7
	MW	ND	181	93	7.7	7
	IW	0.84	6.1	53	38.*	2
	IW	110	484	97	18.*	2
Chloropropylate	MW	ND	10.0	78	8.6	6
	MW	ND	50.0	96	3.3	7
Dibromochloro-propane	MW	ND	1.9	83	12.4	7
	MW	ND	24	70	6.5	7
	IW	ND	1.9	61	--	1
	IW	ND	24	55	1.2*	2
Etridiazole	MW	ND	0.50	144	9.9	7
	MW	ND	9.9	91	1.7	7
PCNB	MW	ND	1.0	100	11.0	7
	MW	ND	20.0	91	3.1	7
Propachlor	IW	21.3	179	87	3.8	7
	MW	ND	895	83	3.8	7

ND = Not detected
MW = Municipal wastewater
IW = Industrial wastewater, pesticide manufacturing

*For duplicate analyses range is listed.

TABLE 3

DISTRIBUTION OF CHLORINATED PESTICIDES
INTO FLORISIL COLUMN FRACTIONS

Parameter	Percent Recovery by Fraction		
	Fraction 1	Fraction 2	Fraction 3
Chlorobenzilate	0	15	70
Chloroneb	93		
Chloropropylate	0	32	61
Etridiazole	100		

Eluant composition by fraction:
Fraction 1 - 200 mL of 6% ethyl ether in hexane
Fraction 2 - 200 mL of 15% ethyl ether in hexane
Fraction 3 - 200 mL of 50% ethyl ether in hexane

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF ETHION IN WASTEWATER

METHOD 614

1. Scope and Application

1.1 This method covers the determination of certain organophosphorus pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Azinphos methyl	39580	86-50-0
Demeton	39560	8065-48-3
Diazinon	39570	333-41-5
Disulfoton	39010	298-04-4
Ethion	--	563-12-2
Malathion	39530	121-75-5
Parathion ethyl	39540	56-38-2
Parathion methyl	39600	298-00-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The method detection limit (MDL, defined in Section 15) for several parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 617. Thus, a single sample may be extracted to measure the parameters included in the scope of both of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under Gas Chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with 15% methylene chloride in hexane using a separatory funnel. The extract is dried and concentrated to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by flame photometric or thermionic bead gas chromatography.

2.2 Method 614 represents an editorial revision of a previously promulgated U.S. EPA method for organophosphorus pesticides.[1] While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.

2.3 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the

heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [3-5] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however,

the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 125-mL, 1000-mL and 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with coarse fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Pipet, disposable - 140 mm long x 5 mm ID.

5.2.9 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required

accessories including syringes, analytical columns, gases, detector and stripchart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 4 mm ID glass, packed with 3% OV-1 on Gas Chrom Q (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 4 mm ID glass, packed with 1.5% OV-17/1.95% QF-1 on Gas Chrom Q (100/120 mesh) or equivalent.

5.6.3 Detector - Phosphorus specific: Flame photometric (FPD) (526 nm filter) or thermionic bead detector in the nitrogen mode. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope. The FPD was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, isooctane, methylene chloride - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Acetonitrile, hexane-saturated - Mix pesticide quality acetonitrile with an excess of hexane until equilibrium is established.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.6 Sodium chloride solution, saturated - Prepare saturated solution of NaCl in reagent water and extract with hexane to remove impurities.

6.7 Alumina - Woelm, neutral; deactivate by pipeting 1 mL of distilled water into a 125-mL ground glass-stoppered Erlenmeyer flask. Rotate flask to distribute water over surface of glass. Immediately add 19.0 g fresh alumina through small powder funnel. Shake flask containing mixture for 2 h on a mechanical shaker.

6.8 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. Before use activate each batch at least 16 h at 130°C in a foil covered glass container.

6.9 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.9.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality isooctane or acetone and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.9.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.9.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with

isooctane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard.

Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value is suggested. This procedure[6] determines the adsorption from hexane solution of lauric acid, in mg, per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absense of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Table 2 provides single operator recovery and precision for diazinon, parathion methyl and parathion ethyl. Similar results should be expected from reagent water for all organophosphorus compounds listed in this method. Compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[7]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL 15% methylene chloride in hexane (V:V) to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL Erlenmeyer flask. Return the aqueous phase to the separatory funnel.

10.3 Add a second 60-mL volume of 15% methylene chloride in hexane to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the 250-mL Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of hexane to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 80 to 85°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of

distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

11.2 Acetonitrile partition - The following acetonitrile partitioning procedure may be used to isolate fats and oils from the sample extracts. The applicability of this procedure to organophosphorus pesticides is indicated in Table 3.

11.2.1 Quantitatively transfer the previously concentrated extract to a 125-mL separatory funnel with enough hexane to bring the final volume to 15 mL. Extract the sample four times by shaking vigorously for 1 min with 30-mL portions of hexane-saturated acetonitrile.

11.2.2 Combine and transfer the acetonitrile phases to a 1-liter separatory funnel and add 650 mL of reagent water and 40 mL of saturated sodium chloride solution. Mix thoroughly for 30 to 45 s. Extract with two 100-mL portions of hexane by vigorously shaking for 15 s.

11.2.3 Combine the hexane extracts in a 1-liter separatory funnel and wash with two 100-mL portions of reagent water. Discard the water layer and pour the hexane layer through a drying column containing 7 to 10 cm of anhydrous sodium sulfate

into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the separatory funnel and column with three 10-mL portions of hexane.

11.2.4 Concentrate the extracts to 6 to 10 mL in the K-D as directed in Section 10.6. Adjust the extract volume to 10 mL with hexane.

11.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

11.3 Florisil column cleanup - The following Florisil column cleanup procedure has been demonstrated to be applicable to the seven organophosphorus pesticides listed in Table 3. It should also be applicable to the cleanup of extracts for ethion.

11.3.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.3.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.3.3 Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2), into a second K-D flask. Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3) and a final elution with 200 mL of 100% ethyl ether (Fraction 4), into separate K-D flasks. The elution patterns for seven of the pesticides are shown in Table 3.

11.3.4 Concentrate the eluates by standard K-D techniques (Section 10.6), using the water bath at about 85°C (75°C for Fraction 4). Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

11.4 Removal of sulfur (9) - Elemental sulfur will elute in Fraction 1 of the Florisil cleanup procedure. If a large amount of sulfur is present in the extract, it may elute in all fractions. If so each fraction must be further treated to remove the sulfur.

11.4.1 Add 1 or 2 boiling chips to the 10-mL hexane solution contained in a concentrator tube. Attach a micro-Snyder column and concentrate the extract to about 0.2 mL in a hot water bath at 85°C. Remove the micro K-D from the bath, cool, and adjust the volume to 0.5 mL with hexane.

11.4.2 Plug a disposable pipet with a small quantity of glass wool. Add enough alumina to produce a 3-cm column after settling. Top the alumina with a 0.5-cm layer of anhydrous sodium sulfate.

11.4.3 Quantitatively transfer the concentrated extract to the alumina microcolumn using a 100-µL syringe. Rinse the ampul with 200-µL of hexane and add to the microcolumn.

11.4.4 Elute the microcolumn with 3 mL of hexane and discard the eluate.

11.4.5 Elute the column with 5 mL of 10% hexane in methylene chloride, and collect the eluate in a 10-mL concentrator tube. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[10] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[11]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[12]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[13] The MDL concentrations listed in Table 1 were obtained using reagent water.[14]

15.2 In a single laboratory, Susquehanna University, using spiked tap water samples, the average recoveries presented in Table 3 were obtained. The standard deviation of the percent recovery is also included in Table 3.[14]

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention Time (min.) Column 1	Column 2	Method Detection Limit (µg/L)
Diazinon	1.8	1.8	0.012
Disulfoton	1.9	2.1	ND
Demeton	2.3	2.1	ND
Parathion methyl	2.5	3.7	0.012
Malathion	2.9	3.9	ND
Parathion ethyl	3.1	4.5	0.015
Ethion	5.8	9.1	ND
Azinphos methyl	14.5	29.9	ND

ND = Not determined

Column 1 conditions: Gas-Chrom Q (100/120 mesh) coated with 3% OV-1 packed in a 1.8 m long x 4 mm ID glass column with nitrogen carrier gas at a flow rate of 60 mL/min. Column temperature, isothermal at 200°C. A flame photometric detector was used with this column to determine the MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 1.5% OV-17+1.95% QF-1 packed in a 1.8 m long x 4 mm ID glass column with nitrogen carrier gas at 70 mL/min flow rate. Column temperature, isothermal at 215°C.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Average Percent Recovery	Standard Deviation (%)	Spike Range (µg/L)	Number of Analyses	Matrix Types
Diazinon	94	5.2	0.04-40	27	4
Parathion methyl	95	3.2	0.06-60	27	4
Parathion ethyl	102	4.1	0.07-70	27	4

TABLE 3

FLORISIL FRACTIONATION PATTERNS
AND ACETONITRILE PARTITION APPLICABILITY

Parameter	Florisil Fractionaction Pattern Percent Recovery by Fraction				Acetonitrile Partition Applicability
	No. 1	No. 2	No. 3	No. 4	
Demeton	100				ND
Disulfoton	100				ND
Diazinon		100			Yes
Malathion		5	95		Yes
Parathion ethyl		100			Yes
Parathion methyl		100			Yes
Azinphos methyl			20	80	ND
Ethion	ND	ND	ND	ND	Yes

ND = Not determined
Florisil eluate composition by fraction
Fraction 1 - 200 mL of 6% ethyl ether in hexane
Fraction 2 - 200 mL of 15% ethyl ether in hexane
Fraction 3 - 200 mL of 50% ethyl ether in hexane
Fraction 4 - 200 mL of ethyl ether

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF 2,4-DB, 2,4-DB ISOBUTYL ESTER (2,4-DB IBE), AND 2,4-DB ISOOCTYL ESTER (2,4-DB IOE), AND DINOSEB IN WASTEWATER

METHOD 615

1. Scope and Application

1.1 This method covers the determination of certain chlorinated herbicides and their esters. The following parent acids can be determined by this method:

Parameter	STORET No.	CAS No.
2,4-D	39736	94-75-7
Dalapon	--	75-99-0
2,4-DB	--	94-82-6
Dicamba	--	1918-00-9
Dichlorprop	--	120-36-5
Dinoseb	--	88-85-7
MCPA	--	94-74-6
MCPP	--	93-65-2
2,4,5-T	39740	93-76-5
2,4,5-TP (Silvex)	39760	93-72-1

1.2 This method is also applicable to the determination of salts and esters of these compounds. This includes, but is not limited to: the isobutyl and isooctyl esters of 2,4-D; the isobutyl and isooctyl esters of 2,4-DB; the isooctyl ester of MCPA; and the isooctyl ester of 2,4,5-TP. The actual form of each acid is not distinguished by this method. Results are calculated and reported for each listed parameter as total free acid.

1.3 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.4 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the

interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for alternative gas chromatographic columns that can be used to confirm measurements made with the primary column. Section 15 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 liter, is acidified. The acid herbicides and their esters and salts are extracted with ethyl ether using a separatory funnel. The derivatives are hydrolyzed with potassium hydroxide and extraneous organic material is removed by a solvent wash. After acidification, the acids are exracted and converted to their methyl esters using diazomethane as the derivatizing agent. Excess reagent is removed, and the esters are determined by electron capture (EC) gas chromatography.[1]

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 The acid forms of the herbicides are strong organic acids, which

react readily with alkaline substances and can be lost during analysis. Glassware and glass wool must be acid-rinsed with (1+9) hydrochloric acid and the sodium sulfate must be acidified with sulfuric acid prior to use to avoid this possibility.

3.3 Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. Alkaline hydrolysis and subsequent extraction of the basic solution remove many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.

3.4 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [3-5] for the information of the analyst.

4.2 Diazomethane is a toxic carcinogen and can explode under certain conditions. The following precautions must be followed:

4.2.1 Use only a well ventilated hood - do not breath vapors.

4.2.2 Use a safety screen.

4.2.3 Use mechanical pipetting aides.

4.2.4 Do not heat above 90°C - EXPLOSION may result.

4.2.5 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers - EXPLOSION may result.

4.2.6 Store away from alkali metals - EXPLOSION may result.

4.2.7 Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride,

and boiling chips.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnels - 60-mL and 2000-mL, with TFE-fluorocarbon stopcocks, ground glass or TFE stoppers.

5.2.2 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.3 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.4 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.5 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.6 Erlenmeyer flask - Pyrex, 250 mL with 24/40 ground glass joint.

5.2.7 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Diazomethane generator - assemble from two 20 x 150 mm test tubes, two Neoprene rubber stoppers and a source of nitrogen. The generator assembly is shown in Figure 1.

5.7 Glass wool - Acid washed (Supelco 2-0383 or equivalent).

5.8 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.8.1 Column 1 - 180 cm long x 4 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 16. Alternative columns may be used in accordance with the provisions described in Section 13.1.

5.8.2 Column 2 - 180 cm long x 4 mm ID glass, packed with 5% OV-210 on Gas Chrom Q (100/120 mesh) or equivalent.

5.8.3 Column 3 - 180 cm long x 2 mm ID glass, packed with 0.1% SP-1000 on Carbopak C (80/100 mesh) or equivalent.

5.8.3 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 13.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, methanol - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and

other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Sodium sulfate - (ACS) Granular, acidified, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h. Acidify by slurrying 100 g sodium sulfate with enough ethyl ether to just cover the solid. Add 0.1 mL concentrated sulfuric acid and mix thoroughly. Remove the ether under vacuum. Mix 1 g of the resulting solid with 5 mL of reagent water and measure the pH of the mixture. It must be below pH 4. Store at 130°C.

6.5 Hydrochloric acid (1+9) - (ACS) Add one volume of concentrated acid to 9 volumes reagent water.

6.6 Potassium hydroxide solution - 37% aqueous solution (W:V). Dissolve 37 g ACS grade potassium hydroxide pellets in reagent water and dilute to 100 mL.

6.7 Sulfuric acid solution (1+1) - Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.8 Sulfuric acid solution (1+3) - Slowly add 25 mL H_2SO_4 (sp. gr. 1.84) to 75 mL of reagent water. Maintain at 4°C.

6.9 Carbitol - (Diethylene glycol monoethyl ether), ACS. Available from Aldrich Chemical Co.

6.10 Diazald - (N-methyl-N-nitroso-p-toluenesulfonamide), ACS. Available from Aldrich Chemical Co.

6.11 Silicic acid - Chromatographic grade, nominal 100 mesh. Store at 130°C.

6.12 Stock standard solutions (1.00 ug/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.12.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure acids. Dissolve the material in pesticide quality ethyl ether and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.12.2 Transfer the stock standard solutions into Teflon-sealed screw-cap vials. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.12.3 Stock standard solutions must be replaced after one week or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system must be calibrated using the external standard technique.

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare working standards of the free acids at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a 10-mL volumetric flask containing 1.0 mL methanol and diluting to volume with ethyl ether. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Prepare calibration standards by esterification of 1.00 mL volumes of the working standards as described in Section 11. Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass of free acid represented by the injection. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the preparation of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Before using any cleanup procedure, the analyst must process a

series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound (acid or ester) to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the

method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[6] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[6]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 14.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram,

confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[7] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel. Check the pH with wide-range pH paper and adjust to pH less than 2 with sulfuric acid (1+1).

10.2 Add 150 mL ethyl ether to the sample bottle, cap the bottle and shake 30 s to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation or other physical means. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL ground-glass Erlenmeyer flask containing 2 mL of 37% potassium hydroxide solution. Approximately 80 mL of the ethyl ether will remain dissolved in the aqueous phase.

10.3 Add a 50-mL volume of ethyl ether to the sample bottle and repeat the extraction a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Add 15 mL reagent water and 1 or 2 clean boiling chips to the 250-mL flask and attach a three-ball Snyder column. Prewet the Snyder column by adding 1 mL ethyl ether to the top. Place the

apparatus on a hot water bath (60 to 65°C), such that the bottom of the flask is bathed in the water vapor. Although the ethyl ether will evaporate in about 15 min, continue heating for a total of 60 min, beginning from the time the flask is placed on the water bath. Remove the apparatus and let stand at room temperature for at least 10 min.

10.5 Transfer the solution to a 60-mL separatory funnel using 5 to 10 mL of reagent water. Wash the basic solution twice by shaking for one min with 20-mL portions of ethyl ether. Discard the organic phase. The free acids remain in the aqueous phase.

10.6 Acidify the contents of the separatory funnel to pH 2 by adding 2 mL of cold (4°C) sulfuric acid (1+3). Test with pH indicator paper. Add 20 mL ethyl ether and shake vigorously for 2 min. Drain the aqueous layer into the 250-mL Erlenmeyer, then pour the organic layer into a 125-mL Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate. Repeat the extraction twice more with 10-mL aliquots of ethyl ether, combining all solvent in the 125-mL flask. Allow the extract to remain in contact with the sodium sulfate for approximately 2 h.

10.7 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.8 Pour the combined extract through a funnel plugged with acid washed glass wool, and collect the extract in the K-D in concentrator. Use a glass rod to crush any caked sodium sulfate during the transfer. Rinse the Erlenmeyer flask and column with 20 to 30 mL of ethyl ether to complete the quantitative transfer.

10.9 Add 1 to 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL ethyl ether to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.10 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of ethyl ether. A 5-mL syringe is recommended for this operation. Add a fresh boiling chip. Attach a micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of ethyl ether to the top. Place the micro K-D apparatus on the water bath so that the

concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 min. When the apparent volume of liquid reaches 0.5 mL, remove the micro K-D from the bath and allow it to drain and cool. Remove the micro Snyder column and add 0.1 mL of methanol. Rinse the walls of the concentrator tube while adjusting the volume to 1.0 mL with ethyl ether.

11. Esterification of Acids

11.1 Assemble the diazomethane generator (See Figure 1) in a hood using two 20 x 150 mm test tubes. Use neoprene rubber stoppers with holes drilled in them to accommodate glass delivery tubes. The exit tube must be drawn to a point to bubble diazomethane through the sample extract.

11.2 Add 5 mL of ethyl ether to the first test tube. Add 1 mL of ethyl ether, 1 mL of carbitol, 1.5 mL of 37% aqueous KOH, and 0.1 to 0.2 g Diazald to the second test tube. Immediately place the exit tube into the concentrator tube containing the sample extract. Apply nitrogen flow (10 mL/min) to bubble diazomethane through the extract for 10 min or until the yellow color of diazomethane persists.

11.3 Remove the concentrator tube and seal it with a Neoprene or Teflon stopper. Store at room temperature in a hood for 20 min.

11.4 Destroy any unreacted diazomethane by adding 0.1 to 0.2 g silicic acid to the concentrator tube. Allow to stand until the evolution of nitrogen gas has stopped. Adjust the sample volume to 10.0 mL with hexane. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. It is recommended that the methylated extracts be analyzed immediately to minimize any transesterification and other potential reactions that may occur. Analyze by gas chromatography.

11.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

12. Cleanup and Separation

12.1 No cleanup procedures were required to analyze the wastewaters described in Section 16. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

13. Gas Chromatography

13.1 Table 1 summarizes the recommended operating conditions for the

gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved for the methyl esters are shown in Figures 2 to 4. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.2 Calibrate the system daily as described in Section 7.

13.3 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[8] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

13.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

13.5 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

13.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

14. Calculations

14.1 Determine the concentration of individual compounds in the sample. Calculate the amount of free acid injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

14.2 Report results in micrograms per liter as acid equivalent without correction for recovery data. When duplicate and spiked samples

are analyzed, report all data obtained with the sample results.

14.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

15. GC/MS Confirmation

15.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the methyl ester of the acid herbicide. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

15.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[9]

15.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[10]

15.4 To confirm an identification of a compound, the background corrected mass spectrum of the methyl ester must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

15.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

15.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

15.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

15.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

15.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup.

16. Method Performance

16.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[11] The MDL concentrations listed in Table 1 were obtained from reagent water with an electron capture detector.[1]

16.2 In a single laboratory (West Cost Technical Services, Inc.), using reagent water and effluents from publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.[1] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

References

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11. Glaser, J.A. et.al, "Trace Analysis for Wastewaters," Environmental Science & Technology, 15, 1426 (1981).

12. "Determination of Chlorinated Herbicides in Industrial and Municipal Wastewater," Method 615, EPA No. 600/4-82-005, NTIS No. PB82-155995, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter (as methyl ester)	Retention Time (min.) Column 1	Column 2	Column 3	Method Detection Limit µg/L
Dicamba	1.2	1.0	--	0.27
2,4-D	2.0	1.6	--	1.2
2,4,5-TP	2.7	2.0	--	0.17
2,4,5-T	3.4	2.4	--	0.20
2,4-DB	4.1	--	--	0.91
Dalapon	--	--	5.0	5.8
MCPP	3.4	--	--	192
MCPA	4.1	--	--	249
Dichlorprop	4.8	--	--	0.65
Dinoseb	11.2	--	--	0.07

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long x 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 70 mL/min. Column temperature: isothermal at 185°C, except for MCPP, MCPA, dichlorprop and dinoseb, where the column temperature was held at 140°C for 6 min and then programmed to 200°C at 10°/min. An electron capture detector was used to measure MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 5% OV-210 packed in a 1.8 m long x 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 70 mL/min. Column temperature: isothermal at 185°C.

Column 3 conditions: Carbopak C (80/100 mesh) coated with 0.1% SP-1000 packed in a 1.8 m long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 25 mL/min. Column temperature: programmed at injection from 100°C to 150°C at 10°/min.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION*

Parameter	Sample Type	Spike (µg/L)	Mean Recovery (%)	Standard Deviation (%)
2,4-D	DW	10.9	75	4
	MW	10.1	77	4
	MW	200	65	5
Dalapon	DW	23.4	66	8
	MW	23.4	96	13
	MW	468	81	9
2,4-DB	DW	10.3	93	3
	MW	10.4	93	3
	MW	208	77	6
Dicamba	DW	1.2	79	7
	MW	1.1	86	9
	MW	22.2	82	6
Dichlorprop	DW	10.7	97	2
	MW	10.7	72	3
	MW	213	100	2
Dinoseb	MW	0.5	86	4
	MW	102	81	3
MCPA	DW	2020	98	4
	MW	2020	73	3
	MW	21400	97	2
MCPP	DW	2080	94	4
	MW	2100	97	3
	MW	20440	95	2
2,4,5-T	DW	1.1	85	6
	MW	1.3	83	4
	MW	25.5	78	5
2,4,5-TP	DW	1.0	88	5
	MW	1.3	88	4
	MW	25.0	72	5

*All results based upon seven replicate analyses.

DW = Reagent water
MW = Municipal water

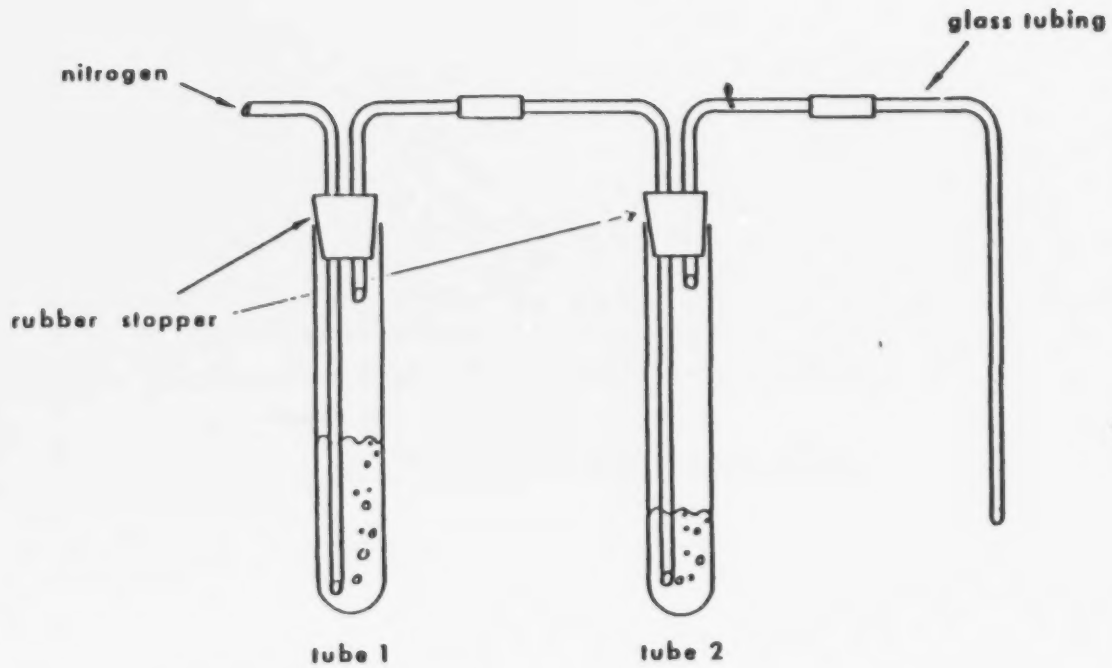

Figure 1. Diazomethane generator.

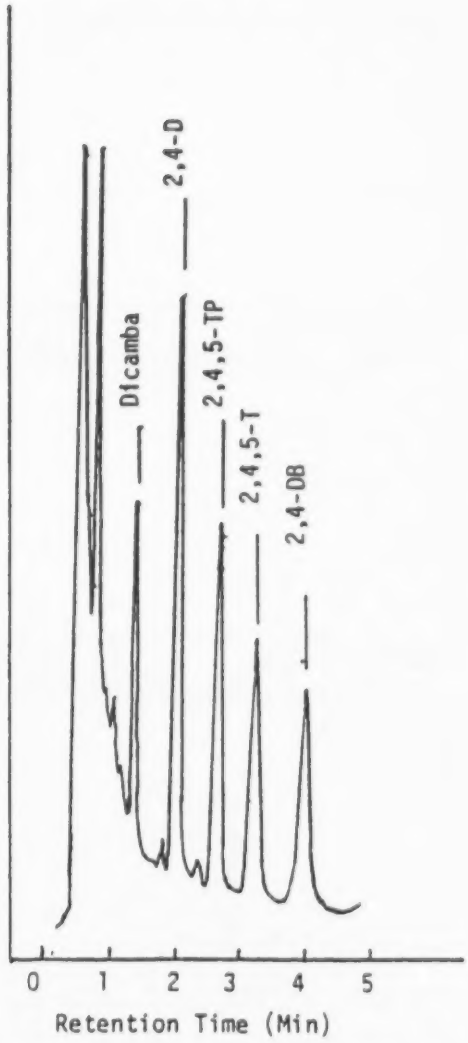

Figure 2. Gas chromatogram of methyl esters of chlorinated herbicides on Column 1. For conditions, see Table 1.

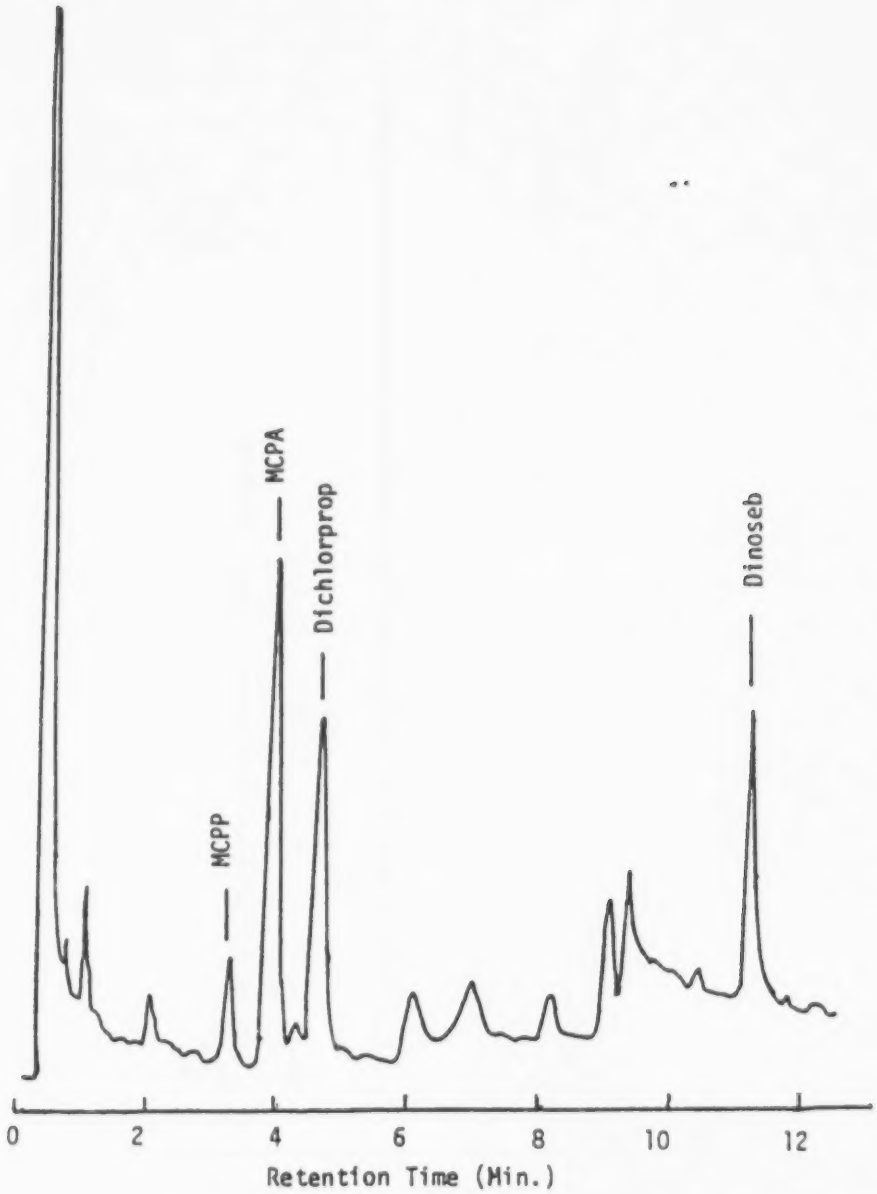

Figure 3. Gas chromatogram of methyl esters of chlorinated herbicides on Column 1. For conditions, see Table 1.

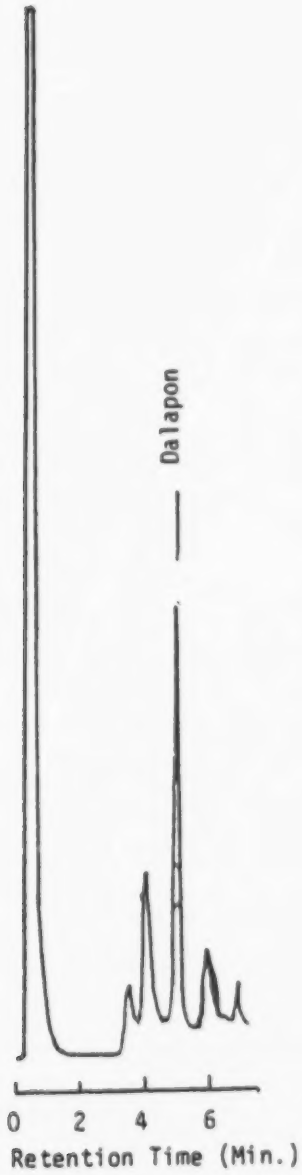

Figure 4. Gas chromatogram of methyl ester of dalapon on Column 3. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF CARBOPHENOTHION IN WASTEWATER

METHOD 617

1. Scope and Application

1.1 This method covers the determination of certain organohalide pesticides and PCBs. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Aldrin	39330	309-00-2
α-BHC	39337	319-84-6
β-BHC	39338	319-85-7
δ-BHC	39259	319-86-8
γ-BHC (Lindane)	39340	58-89-9
Captan	39640	133-06-2
Carbophenothion	-----	786-19-6
Chlordane	39350	5103-74-2
4,4'-DDD	39310	72-54-8
4,4'-DDE	39320	72-55-9
4,4'-DDT	39300	50-29-3
Dichloran	-----	99-30-9
Dicofol	39780	115-32-2
Dieldrin	39380	60-57-1
Endosulfan I	34356	959-98-8
Endosulfan II	34361	33213-65-9
Endosulfan sulfate	34351	1031-07-8
Endrin	39390	72-20-8
Endrin aldehyde	34366	7421-93-4
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Isodrin	39430	465-73-6
Methoxychlor	39480	72-43-5
Mirex	39755	2385-85-5
PCNB	39029	82-68-8
Perthane	39034	72-56-0
Strobane	-----	8001-50-1
Toxaphene	39400	8001-35-2
Trifluralin	39030	1582-09-8
PCB-1016	34671	12674-11-2
PCB-1221	39488	11104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9

Parameter	STORET No.	CAS No.
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The method detection limit (MDL, defined in Section 15) for many of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 614. Thus, a single sample may be extracted to measure the parameters included in the scope of both of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under Gas Chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with 15% methylene chloride in hexane using a separatory funnel. The extract is dried and concentrated to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture gas chromatography.

2.2 Method 617 represents an editorial revision of two previously promulgated U.S. EPA methods for pesticides and for PCBs.[1] While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.

2.3 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large late eluting peaks, especially in the 15 and 50% fractions from the Florisil column cleanup. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.[3,4] The interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.

3.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: aldrin, benzene hexachlorides, chlordane, heptachlor, PCNB, PCBs and toxaphene. Primary standards of these toxic materials should be prepared in a hood.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 125-mL, 1000-mL and 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with coarse fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Shaker - Laboratory, reciprocal action.

5.7 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and stripchart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 4 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 4 mm ID glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, isooctane, methylene chloride - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Acetonitrile, hexane-saturated - Mix pesticide quality acetonitrile with an excess of hexane until equilibrium is established.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.6 Sodium chloride solution, saturated - Prepare saturated solution of NaCl in reagent water and extract with hexane to remove impurities.

6.7 Sodium hydroxide solution (10N) - Dissolve 40 g ACS grade NaOH in reagent water and dilute to 100 mL.

6.8 Sulfuric acid solution (1+1) - Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.9 Mercury - Triple distill.

6.10 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. Before use activate each batch at least 16 h at 130°C in a foil covered glass container.

6.11 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.11.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality isooctane and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.11.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.11.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration

factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value is suggested. This procedure[8] determines the adsorption from hexane solution of lauric acid, in mg, per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

7.6 The multipeak materials included in this method present a special calibration problem. Recommended procedures for calibration, separation and measurement of PCBs is discussed in detail in the previous edition of this method.[1] Illustrated methods for the calibration and measurement of chlordane and strobane/toxaphene are available elsewhere.[9]

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Table 2 provides single operator recovery and precision for many of the organohalide pesticides. Similar results should be expected from reagent water for all parameters listed in this method. Compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[10] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[10]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[11] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid. Record the volume of acid or base used.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL 15% methylene chloride in hexane (V:V) to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL Erlenmeyer flask. Return the aqueous phase to the separatory funnel.

10.3 Add a second 60-mL volume of 15% methylene chloride in hexane to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the 250-mL Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of hexane to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 80 to 85°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

11.2 Acetonitrile partition - The following acetonitrile partitioning procedure may be used to isolate fats and oils from the sample extracts. This procedure is applicable to all of the parameters in this method except mirex.

11.2.1 Quantitatively transfer the previously concentrated extract to a 125-mL separatory funnel with enough hexane to bring the final volume to 15 mL. Extract the sample four times by shaking vigorously for 1 min with 30-mL portions of hexane-saturated acetonitrile.

11.2.2 Combine and transfer the acetonitrile phases to a 1-liter separatory funnel and add 650 mL of reagent water and 40 mL of saturated sodium chloride solution. Mix thoroughly for 30 to 45 s. Extract with two 100-mL portions of hexane by vigorously shaking for 15 s.

11.2.3 Combine the hexane extracts in a 1-liter separatory funnel and wash with two 100-mL portions of reagent water. Discard the water layer and pour the hexane layer through a drying column containing 7 to 10 cm of anhydrous sodium sulfate into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the separatory funnel and column with three 10-mL portions of hexane.

11.2.4 Concentrate the extracts to 6 to 10 mL in the K-D as directed in Section 10.6. Adjust the extract volume to 10 mL with hexane.

11.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

11.3 Florisil column cleanup - The following Florisil column cleanup procedure has been demonstrated to be applicable to most of the organochlorine pesticides and PCBs listed in Table 3. It should also be applicable to the cleanup of extracts for PCNB, strobane and trifluralin.

11.3.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.3.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.3.3 Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2), into a second K-D flask. Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3) into a separate K-D flask. The elution patterns for the pesticides and PCBs are shown in Table 3.

11.3.4 Concentrate the eluates by standard K-D techniques (Section 10.6), using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

11.4 Removal of sulfur - Elemental sulfur will elute in Fraction 1 of the Florisil cleanup procedure. If a large amount of sulfur is present in the extract, it may elute in all fractions. If so, each fraction must be further treated to remove the sulfur. This procedure cannot be used with heptachlor, endosulfans, or endrin aldehyde.

11.4.1 Pipet 1.00 mL of the concentrated extract into a clean concentrator tube or a vial with a TFE-fluorocarbon seal. Add 1 to 3 drops of mercury and seal.

11.4.2 Agitate the contents of the vial for 15 to 30 s.

11.4.3 Place the vial in an upright position on a reciprocal laboratory shaker and shake for up to 2 h.

11.4.4 If the mercury appears shiny after this treatment, analyze the extract by gas chromatography. If the mercury is black, decant the extract into a clean vial and repeat the cleanup with fresh mercury.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[12] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used. Multipeak materials present a special analytical problem beyond the scope of this discussion. Illustrated procedures for calibration and measurement are available for PCBs[1] and pesticides.[9]

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{A}{\left(\frac{V_i}{V_t}\right)\left(V_s\right)}$$

where:

A = Amount of material injected in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface

constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[13]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[14]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[15] The MDL concentrations listed in Table 1 were obtained using reagent water.[16]

15.2 In a single laboratory, Susquehanna University, using spiked tap water samples, the average recoveries presented in Table 2 were obtained. The standard deviation of the percent recovery is also included in Table 2.[16]

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TABLE 1

GAS CHROMATOGRAPHY OF PESTICIDES AND PCBs*

Parameter	Retention Time (min) Column 1	Column 2	Method Detection Limit (ug/L)
Aldrin	2.40	4.10	0.009
α-BHC	1.35	1.82	0.004
β-BHC	1.90	1.97	ND
δ-BHC	2.15	2.20	ND
γ-BHC	1.70	2.13	0.002
Captan	6.22	5.00	ND
Carbophenothion	10.90	10.90	ND
4,4'-DDD	7.83	9.08	0.012
4,4'-DDE	5.13	7.15	0.004
4,4'-DDT	9.40	11.75	0.032
Dichloran	1.85	2.01	ND
Dicofol	2.86	4.59	ND
Dieldrin	5.45	7.23	0.011
Endosulfan I	4.50	6.20	0.011
Endosulfan II	8.00	8.28	0.017
Endosulfan sulfate	14.22	10.70	ND
Endrin	6.55	8.10	ND
Endrin aldehyde	11.82	9.30	ND
Heptachlor	2.00	3.35	0.004
Heptachlor epoxide	3.50	5.00	0.003
Isodrin	3.00	4.83	ND
Methoxychlor	18.20	26.60	0.176
Mirex	14.60	15.50	0.015
PCNB	1.63	2.01	0.002
Trifluralin	0.94	1.35	0.013

*For multipeak materials, see Figures 2 to 10 for chromatographic conditions and retention patterns.

ND = Not Determined.

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 in a 1.8 m long x 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 60 mL/min. Column temperature: isothermal at 200°C. An electron capture detector was used with this column to determine the MDL.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 packed in a 1.8 m long x 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 60 mL/min. Column temperature: isothermal at 200°C.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION
FOR TAP WATER

Parameter	Average Percent Recovery	Standard Deviation (%)	Spike Range (µg/L)	Number of Analyses
Aldrin	78.1	5.4	0.03-3.0	21
δ-BHC	95.3	8.9	0.01-1.0	21
γ-BHC	95.1	7.2	0.01-1.0	21
4,4'-DDD	94.4	5.0	0.08-8.0	21
4,4'-DDE	89.8	3.7	0.05-5.0	21
4,4'-DDT	91.0	4.5	0.2-20	21
Dieldrin	98.2	4.9	0.06-6.0	21
Endosulfan I	101.0	7.6	0.05-5.0	21
Endosulfan II	92.9	4.8	0.09-9.0	21
Heptachlor	84.4	6.4	0.02-2.0	21
Heptachlor epoxide	93.7	3.9	0.03-3.0	21
Methoxychlor	96.6	6.7	0.6-60	21
Mirex	89.1	4.8	0.2-20	21
PCNB	82.6	6.2	0.01-1.0	21
Trifluralin	94.3	10.5	0.03-3.0	21

TABLE 3

DISTRIBUTION AND RECOVERY OF CHLORINATED PESTICIDES AND PCBs USING FLORISIL COLUMN CHROMATOGRAPHY

Parameter	Percent Recovery by Fraction No. 1	No. 2	No. 3
Aldrin	100		
α-BHC	100		
β-BHC	97		
δ-BHC	98		
γ-BHC	100		
Captan	+	+	
Carbofenthion	100		
Chlordane	100		
4,4'-DDD	99		
4,4'-DDE	98		
4,4'-DDT	100		
Dicofol	+	+	
Dieldrin	0	100	
Endosulfan I	37	64	
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	
Endrin aldehyde	0	68	26
Heptachlor	100		
Heptachlor epoxide	100		
Isodrin	100		
Methoxychlor	100		
Mirex	100		
Perthane	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95		
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260	95		

+Compound occurs in both 6% and 15% fractions.
Florisil eluate composition by fraction.
Fraction 1 - 200 mL of 65 ethyl ether in hexane.
Fraction 2 - 200 mL of 15% ethyl ether in hexane.
Fraction 3 - 200 mL of 50% ethyl ether in hexane.

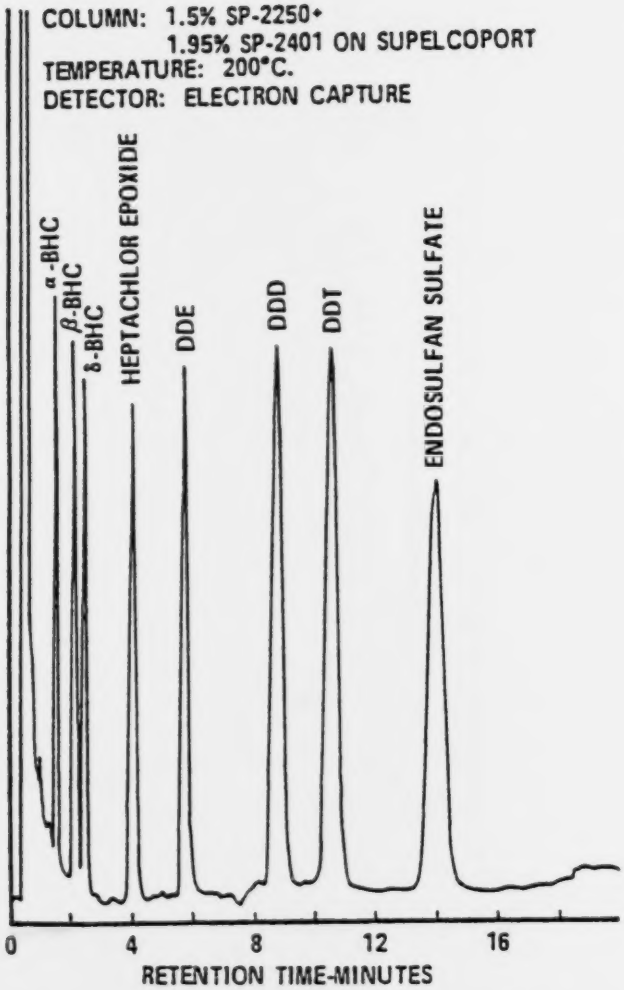

Figure 1. Gas chromatogram of pesticides

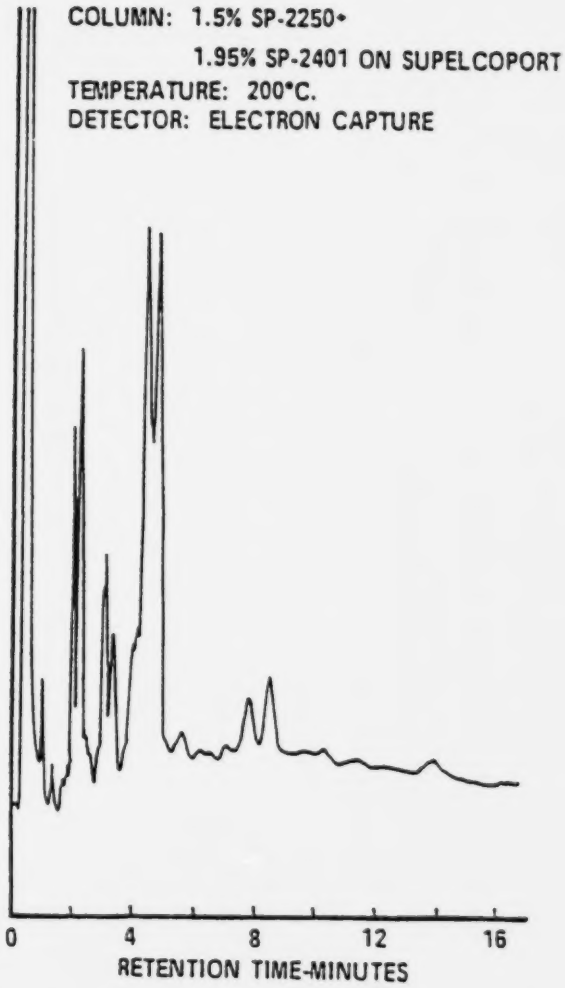

Figure 2. Gas chromatogram of chlordane

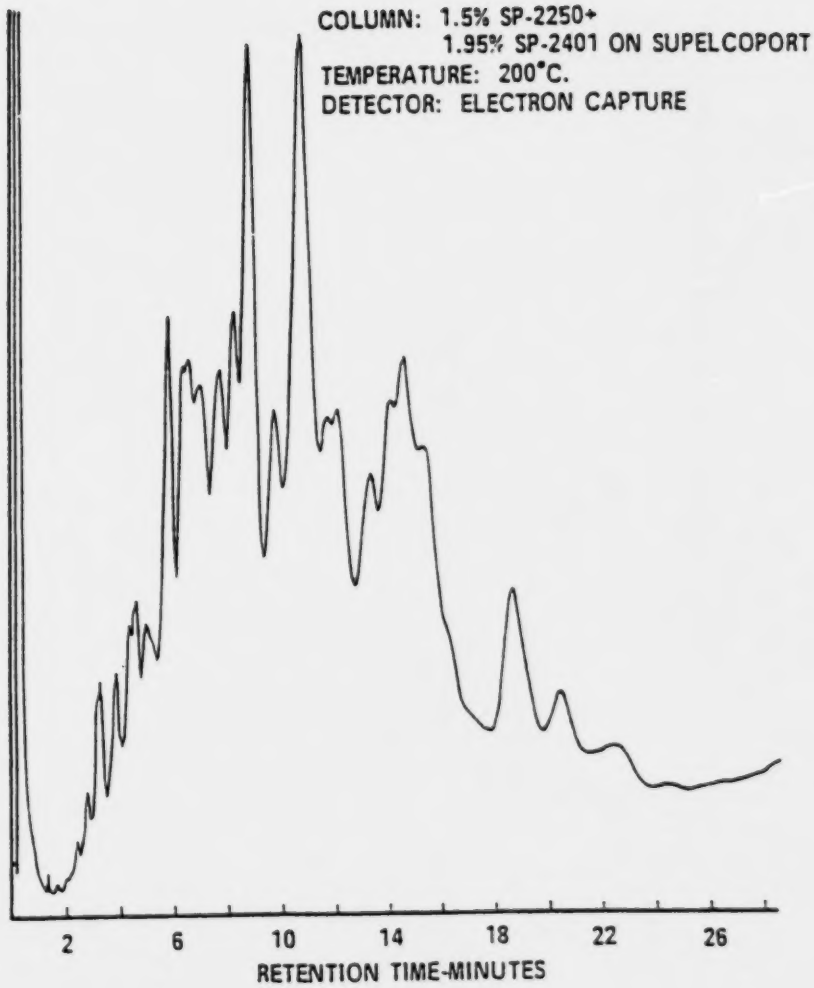

Figure 3. Gas chromatogram of toxaphene

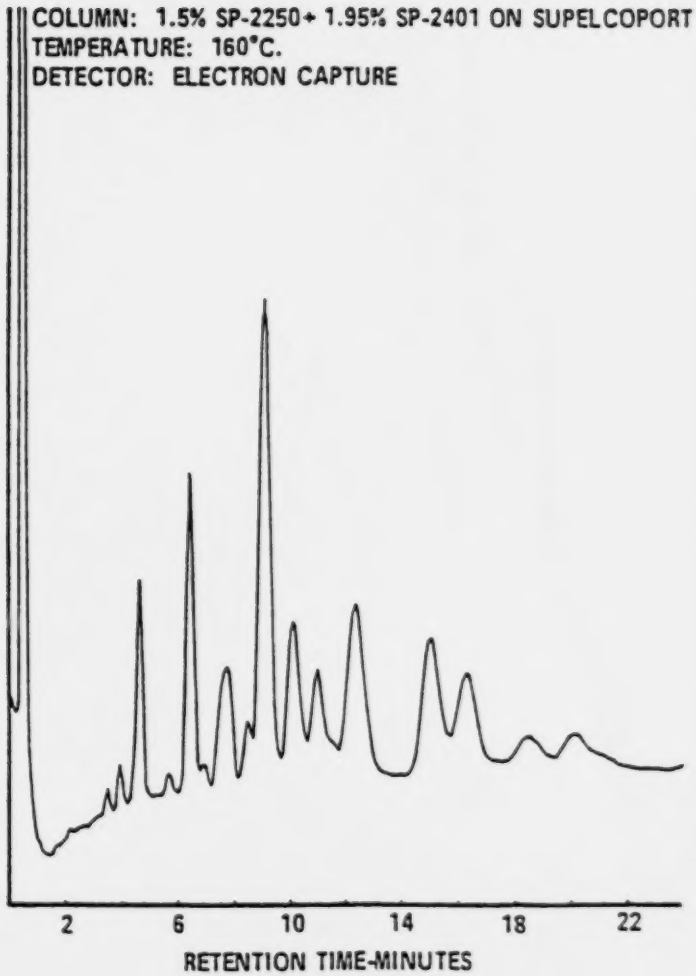

Figure 4. Gas chromatogram of PCB-1016

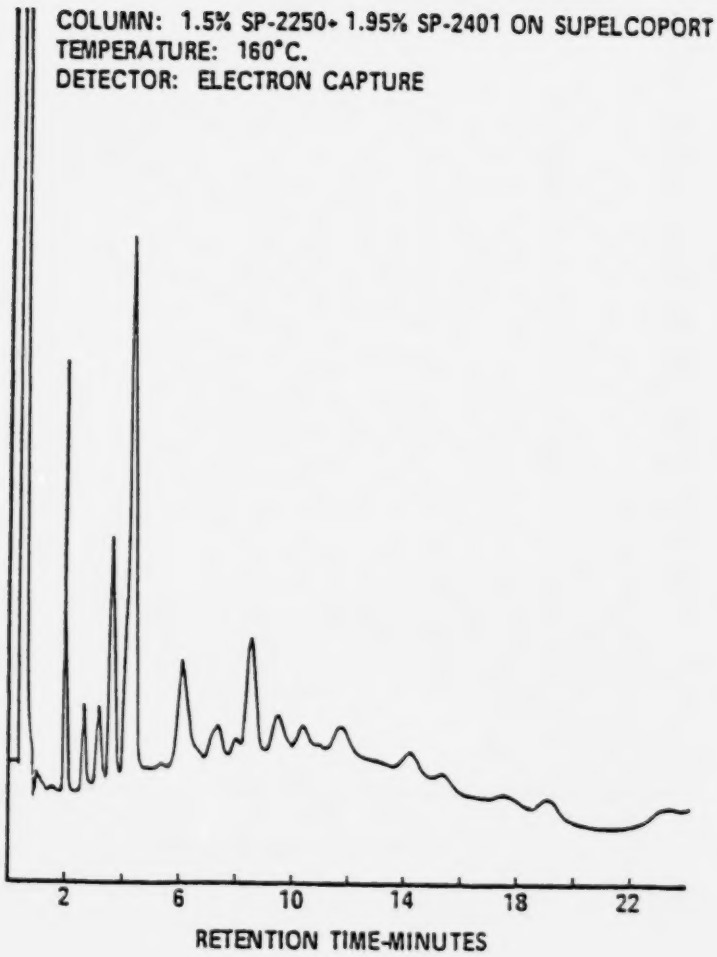

Figure 5. Gas chromatogram of PCB-1221

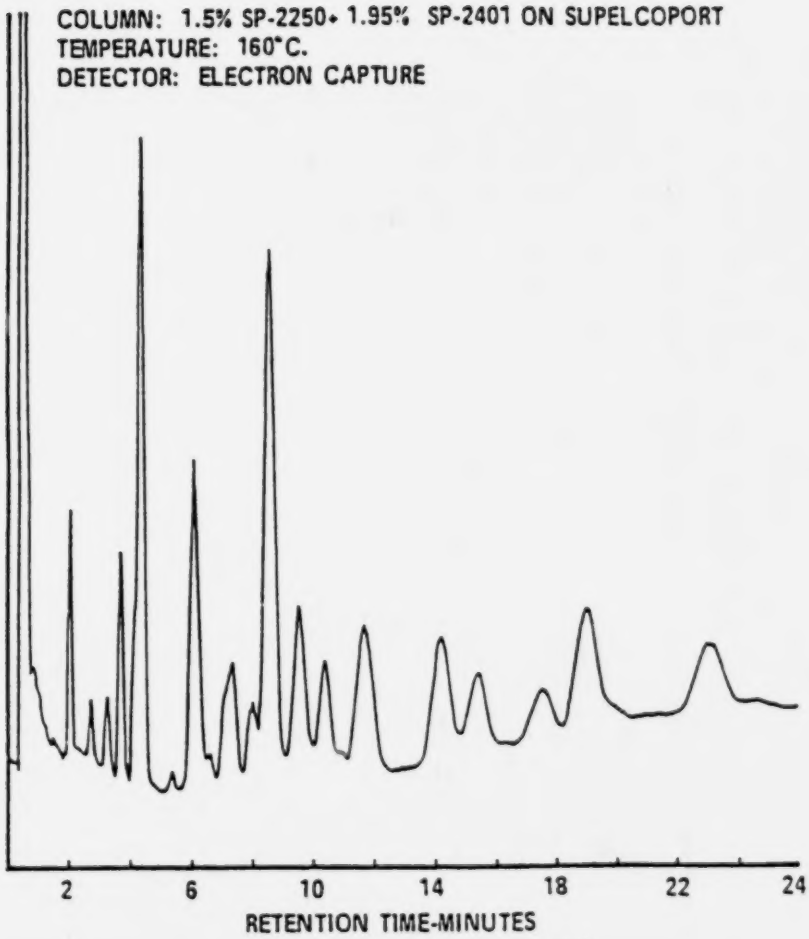

Figure 6. Gas chromatogram of PCB-1232

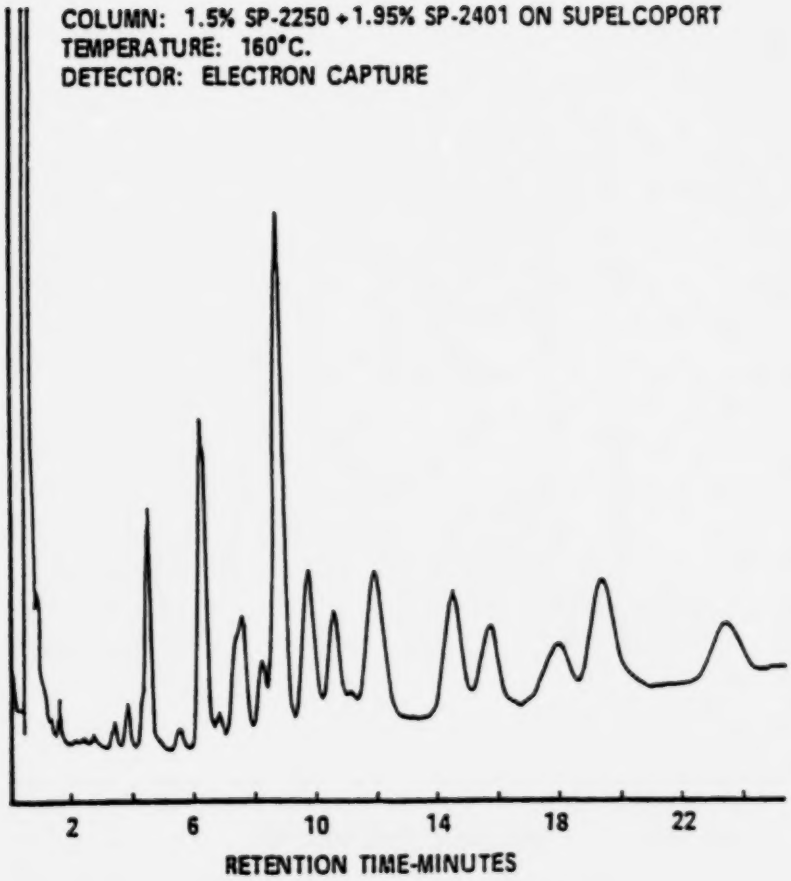

Figure 7. Gas chromatogram of PCB-1242

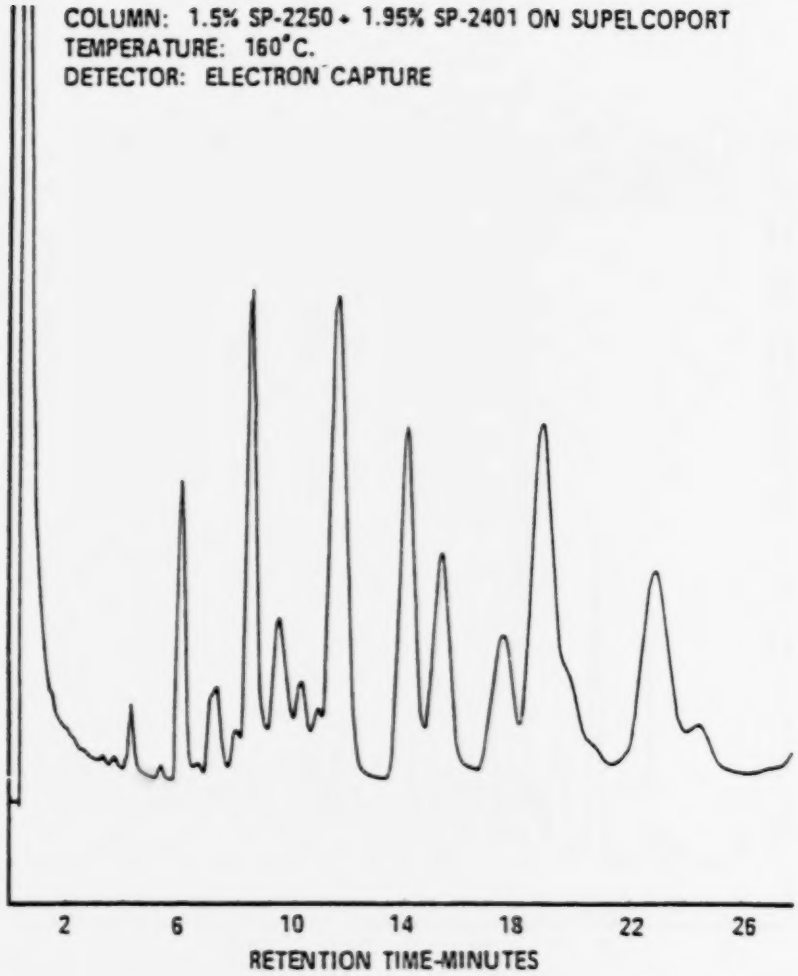

Figure 8. Gas chromatogram of PCB-1248

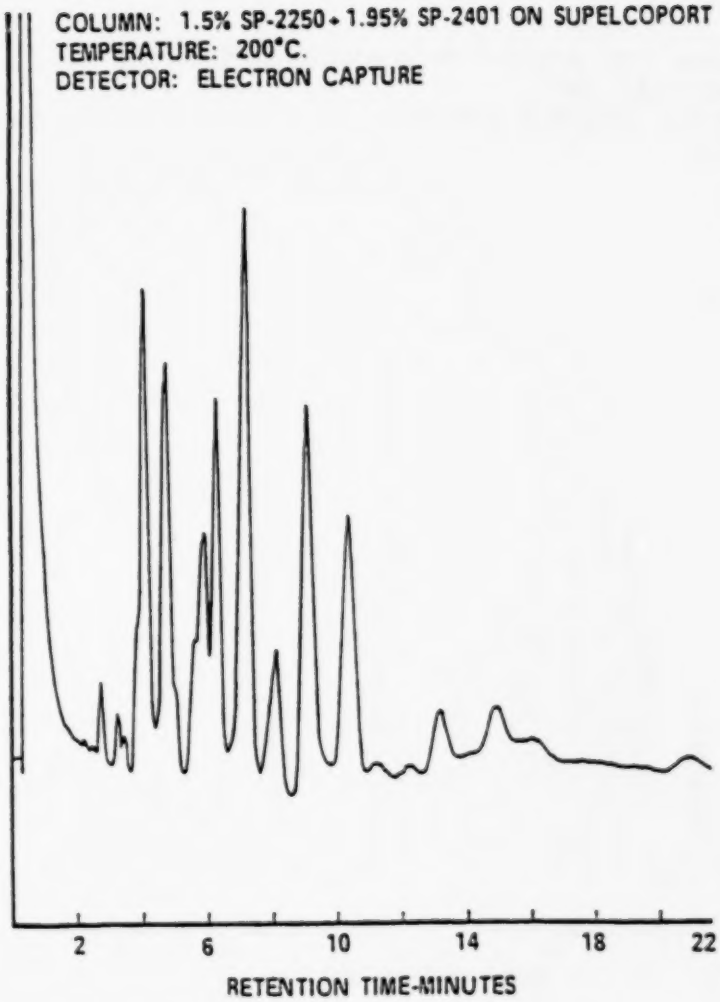

Figure 9. Gas chromatogram of PCB-1254

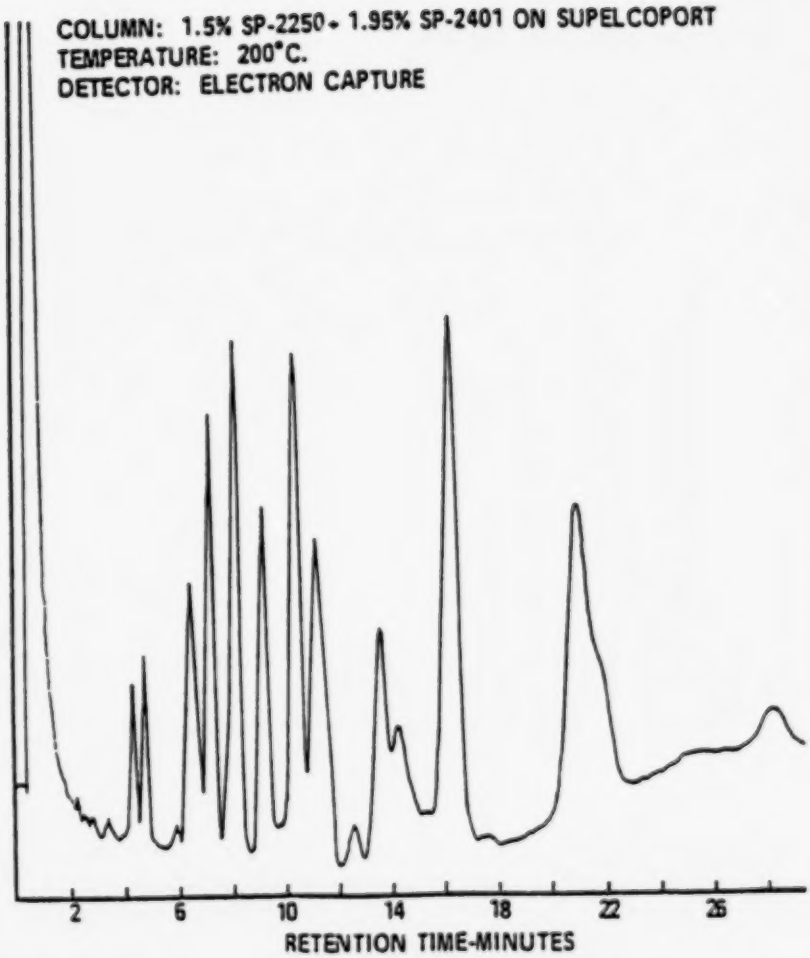

Figure 10. Gas chromatogram of PCB-1260

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF SIMETRYN AND TERBUTRYN IN WASTEWATER

METHOD 619

1. Scope and Application

1.1 This method covers the determination of certain triazine pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Ametryn	--	834-12-8
Atraton	--	1610-17-9
Atrazine	39033	1912-24-9
Prometon	39056	1610-18-0
Prometryn	39057	7287-19-6
Propazine	39024	139-40-2
Secbumeton	--	26259-45-0
Simetryn	39054	1014-70-6
Simazine	39055	122-34-9
Terbuthylazine	--	5915-41-3
Terbutryn	--	886-50-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The estimated method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as several others in the 600-method series. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under Gas Chromatography, the analyst is allowed the latitude to select chromatographic conditions

appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead detector in the nitrogen mode.[1,2]

2.2 Method 619 represents an editorial revision of a previously promulgated U.S. EPA method for organophosphorus pesticides.[3] While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.

2.3 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[4] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle

furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible

silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with coarse fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 5% Carbowax 20M-TPA on Supelcoport (80/100 mesh) or

equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 4 mm ID glass, packed with 1.0% Carbowax 20M on Gas Chrom Q (100/120 mesh) or equivalent.

5.6.3 Detector - Thermionic bead in the nitrogen mode. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, methylene chloride, methanol - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.5 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. Before use activate each batch at least 16 h at 130°C in a foil covered glass container.

6.6 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.6.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality hexane or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the

analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.6.2 Transfer the stock standard solutions into TFE-fluorocarbon sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.6.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%,

the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which

is used, the use of lauric acid value is suggested. This procedure[8] determines the adsorption from hexane solution of lauric acid, in mg, per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard

deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the data from Table 2, estimate the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s

Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[9] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[9]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[10] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. (Note: Precipitation of triazines in the hexane may occur if the concentration in the original sample exceeded 500 µg/L. If this occurs, redissolve the triazines in methylene chloride and analyze the extract using flame ionization gas chromatography.) Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the nine triazine pesticides listed in Table 3.

11.2.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.2.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.2.3 Drain the column until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. This fraction may be discarded. Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Elute the column into the flask, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2). Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3), and a final elution with 200 mL of 100% ethyl ether (Fraction 4), into separate K-D flasks. The elution patterns for nine of the pesticides are shown in Table 3.

11.2.4 Concentrate the eluates by standard K-D techniques (Section 10.6), substituting hexane for the glassware rinses and using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this

method. An example of the separation achieved by Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[11] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[12]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[13]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic

conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[14] The MDL concentrations listed in Table 1 were estimated from the response of the thermionic bead nitrogen detector to each compound. The estimate is based upon the amount of material required to yield a signal five times the GC background noise, assuming a 5-µL injection from a 10-mL final extract of a 1-liter sample.

15.2 In a single laboratory (either West Cost Technical Services, Inc. or Midwest Research Institute), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained after Florisil cleanup.[1,2] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention Time (min.) Column 1	Column 2	Method Detection Limit (µg/L)
Prometon	6.9	4.9	0.03
Atraton	--	6.3	ND
Propazine	9.2	6.7	0.03
Terbuthylazine	10.2	7.3	0.03
Secbumeton	--	8.3	ND
Atrazine	12.4	9.4	0.05
Prometryn	13.8	10.3	0.06
Terbutryn	15.4	--	0.05
Simazine	16.3	12.7	0.06
Ametryn	17.7	14.0	0.06
Simetryn	23.0	--	0.07

ND = Not determined

Column 1 conditions: Supelcoport (80/100 mesh) coated with 5% Carbowax 20M-TPA packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature, isothermal at 200°C. A thermionic bead detector was used with this column to determine the MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 1.0% Carbowax 20 M packed in a 1.8 m long x 4 mm ID glass column with helium carrier gas at 80 mL/min flow rate. Column temperature, isothermal at 155°C.

TABLE 2

SINGLE LABORATORY ACCURACY AND PRECISION

Parameter	Sample Type*	Spike (µg/L)	Number of Replicates	Mean Recovery (%)	Standard Deviation (%)
Ametryn	3	4,000	2	104	--
	3	2,000	2	118	--
Atrazine	3	300	2	108	--
	1	1,000	7	177	15.2
Prometon	1	130	7	67	3.9
	2	260	7	51	3.0
Prometryn	3	2,000	2	76	--
	3	50	2	110	--
Propazine	1	516	7	54	6.5
	3	15	2	116	--
Simatryn	3	30	2	183	--
	3	15	2	182	--
Simazine	1	115	7	152	24.3
	3	10	2	99	--
Terbuthylazine	3	100	2	114	--
	3	15	2	100	--
Terbutryn	1	968	7	83	10.0
	2	169	7	89	24.0

*Sample Type

1 - Industrial process water
2 - Industrial effluent
3 - 80% Industrial process water/20% industrial effluent

TABLE 3

FLORISIL FRACTIONATION PATTERNS

Parameter	Percent Recovery by Fraction			
	No. 1	No. 2	No. 3	No. 4
Propazine	0	90	10	
Terbuthylazine	0	30	70	
Atrazine	0	20	80	
Ametryn			100	
Prometryn			100	
Simazine			100	
Atraton			100	
Secbumeton				100
Prometon				100

Florisil eluate composition by fraction
Fraction 1 - 200 mL of 6% ethyl ether in hexane
Fraction 2 - 200 mL of 15% ethyl ether in hexane
Fraction 3 - 200 mL of 50% ethyl ether in hexane
Fraction 4 - 200 mL of ethyl ether

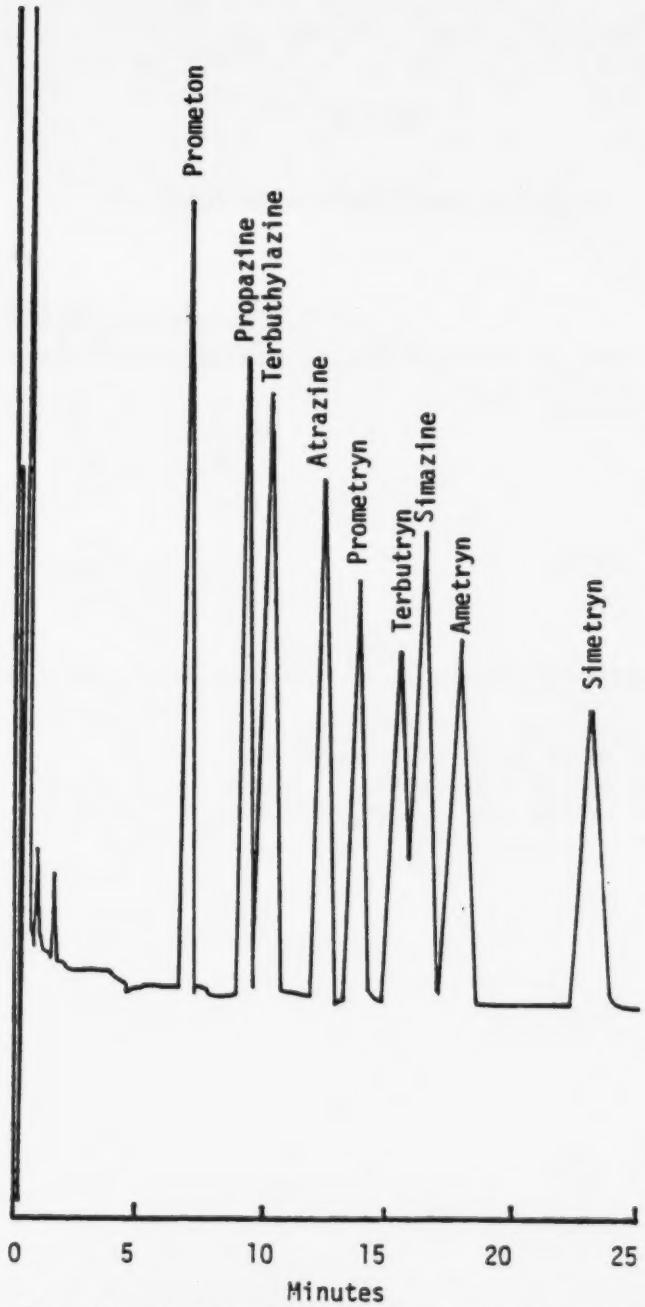

Figure 1. Gas chromatogram of triazine pesticides on Column 1. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BOLSTAR, CHLORPYRIFOS, CHLORPYRIFOS METHYL, COUMAPHOS, DICHLORVOS, FENSULFOTHION, FENTHION, MEVINPHOS, NALED, PHORATE, RONNEL, STIROFOS, AND TRICHLORONATE IN WASTEWATER

METHOD 622

1. Scope and Application

1.1 This method covers the determination of certain organophosphorus pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Azinphos methyl	39580	86-50-0
Bolstar	--	35400-43-2
Chlorpyrifos	--	2921-88-2
Chlorpyrifos methyl	--	5598-13-0
Coumaphos	81293	56-72-4
Demeton	39560	8065-48-3
Diazinon	39570	333-41-5
Dichlorvos	--	62-73-7
Disulfoton	39010	298-04-4
Ethoprop	--	13194-48-4
Fensulfothion	--	115-90-2
Fenthion	39016	55-38-9
Merphos	39019	150-50-5
Mevinphos	--	7786-34-7
Naled	--	300-76-5
Parathion methyl	39600	298-00-0
Phorate	39023	298-02-2
Ronnel	39357	299-84-3
Stirofos	--	961-11-5
Tokuthion	--	34643-46-4
Trichloronate	--	327-98-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The estimated method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific

wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as several others in the 600-method series. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under Gas Chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead or flame photometric detector in the phosphorus mode.[1]

Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle

furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require special clean-up approaches or selective GC detectors to achieve the MDL listed in Table 1. Use of a flame photometric detector in the phosphorus mode will minimize interferences from materials that do not contain phosphorus. Elemental sulfur, however, may interfere with the determination of certain organophosphorus pesticides by flame photometric gas chromatography. A halogen specific detector (electrolytic conductivity or microcoulometric) is very selective for the halogen containing pesticides and has been shown to be effective in the analysis of wastewater for dichlorvos, naled and stirofos.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [3-5] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene

chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Columns - These columns were used to develop the method performance statements in Section 15. Alternate columns may be used in accordance with the provisions described in Section 12.1.

5.6.1.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 5% SP-2401 on Supelcoport (100/120 mesh) or equivalent.

5.6.1.2 Column 2 - 180 cm long x 2 mm ID glass, packed with 3% SP-2401 on Supelcoport (100/120 mesh) or equivalent.

5.6.1.3 Column 3 - 50 cm long x 1/8 inch OD Teflon, packed with 15% SE-54 on Gas Chrom Q (80/100 mesh) or equivalent.

5.6.2 Detector - Thermionic bead or flame photometric in the phosphorus mode. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope and were used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, methylene chloride - Pesticide quality or equivalent.

6.3 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.5 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.5.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality hexane or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared

stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.5.2 Transfer the stock standard solutions into TFE-fluorocarbon sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.6.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s

Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[6] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[6]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform

analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[7] should be followed; however, the bottle must not be preringed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix and were not required for the analysis of the wastewaters reported in Section 15. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Naled is partially converted to dichlorvos on GC Columns 1 and 2 but not on Column 3. Therefore, if naled is to be measured

in the sample, GC analysis for dichlorvos and naled must be performed using Column 3. Examples of the separations achieved are shown in Figures 1 to 4. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[8] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[9]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[10]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic

conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[11] The MDL concentrations listed in Table 1 were estimated from the response of the detector to each compound. The estimate is based upon the amount of material required to yield a signal five times the GC background noise, assuming a 5-µL injection from a 10-mL final extract of a 1-liter sample.

15.2 In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.[1] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND ESTIMATED METHOD DETECTION LIMITS

Parameter	GC Column	Retention Time (Min)	Estimated MDL (µg/L)
Demeton	1a	1.16, 2.53	0.25
Phorate	1a	1.43	0.15
Disulfoton	1a	2.10	0.20
Trichloronate	1a	2.94	0.15
Fenthion	1a	3.12	0.10
Tokuthion	1a	3.40	0.5
Bolstar	1a	4.23	0.15
Fensulfothion	1a	6.41	1.5
Azinphos methyl	1a	6.80	1.5
Coumaphos	1a	11.6	1.5
Dichlorvos	1b	0.8	0.1
Mevinphos	1b	2.41	0.3
Stirofos	1b	8.52	5.0
Ethoprop	2	3.02	0.25
Parathion methyl	2	3.37	0.3
Ronnel	2	5.57	0.3
Chlorpyrifos methyl	2	5.72	0.3
Chlorpyrifos	2	6.16	0.3
Merphos	2	7.45	0.25
Diazinon	2	7.73	0.6
Dichlorvos	3	1.50	0.1
Naled	3	3.28	0.1
Stirofos	3	5.51	5.0

Column 1a Conditions: Supelcoport (100/120 mesh) coated with 5% SP-2401 packed in a 180 cm long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature, programmed: initial 150°C, hold for 1 min, then program at 25°C/min to 220°C and hold. A flame photometric detector was used with this column to estimate the MDL.

Column 1b Conditions: Same as Column 1a, except nitrogen carrier gas at a flow rate of 30 ml/min. Temperature, programmed: initial 170°C, hold 2 min, then program at 20°C/min to 220°C and hold.

Column 2 Conditions: Supelcoport (100/120 mesh) coated with 3% SP-2401 packed in a 180 cm long x 2 mm ID glass column with helium carrier gas at a flow rate of 25 mL/min. Column temperature, programmed, initial 170°C, hold for 7 min, then program at 10°C/min to 250°C and hold. A thermionic bead detector was used with this column to estimate the MDL.

TABLE 1. (Continued)

Column 3 Conditions: Gas Chrom Q (100/120 mesh) coated with 15% SE-54 packed in a 50 cm long x 1/8 in. OD Teflon column with nitrogen carrier gas at a flow rate of 30 mL/min. Temperature, programmed: initial 100°C, then program immediately at 25°C/min to 200°C and hold. An electrolytic detector in the halogen mode was used with this column to estimate the MDL.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Average Percent Recovery	Standard Deviation (%)	Spike Range (µg/L)	Number of Analyses	Matrix Types
Azinphos methyl	72.7	18.8	21-250	17	3
Bolstar	64.6	6.3	4.9-46	17	3
Chlorpyrifos	98.3	5.5	1.0-50.5	18	3
Coumaphos	109.0	12.7	25-225	17	3
Demeton	67.4	10.5	11.9-314	17	3
Diazinon	67.0	6.0	5.6	7	1
Dichlorvos	72.1	7.7	15.6-517	16	3
Disulfoton	81.9	9.0	5.2-92	17	3
Ethoprop	100.5	4.1	1.0-51.5	18	3
Fensulfothion	94.1	17.1	23.9-110	17	3
Fenthion	68.7	19.9	5.3-64	17	3
Merphos	120.7	7.9	1.0-50	18	3
Mevinphos	56.5	7.8	15.5-520	16	3
Naled	78.0	8.1	25.8-294	16	3
Parathion methyl	96.0	5.3	0.5-500	21	3
Phorate	62.7	8.9	4.9-47	17	3
Ronnel	99.2	5.6	1.0-50	18	3
Stirofos	66.1	5.9	30.3-505	16	3
Tokuthion	64.6	6.8	5.3-64	17	3
Trichloronate	105.0	18.6	20	3	1

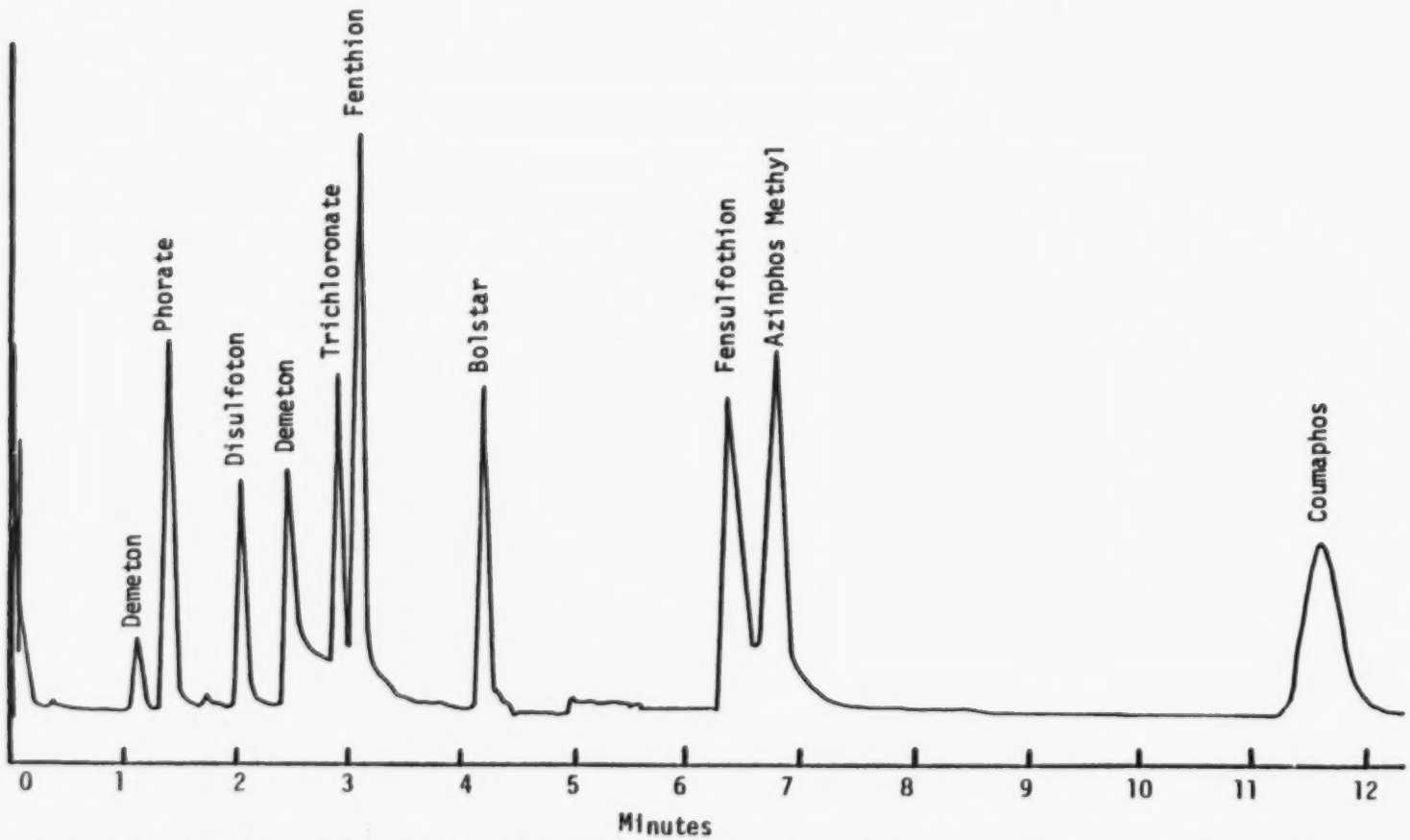

Figure 1. Gas chromatogram of organophosphorus pesticides on Column 1a. For conditions, see Table 1.

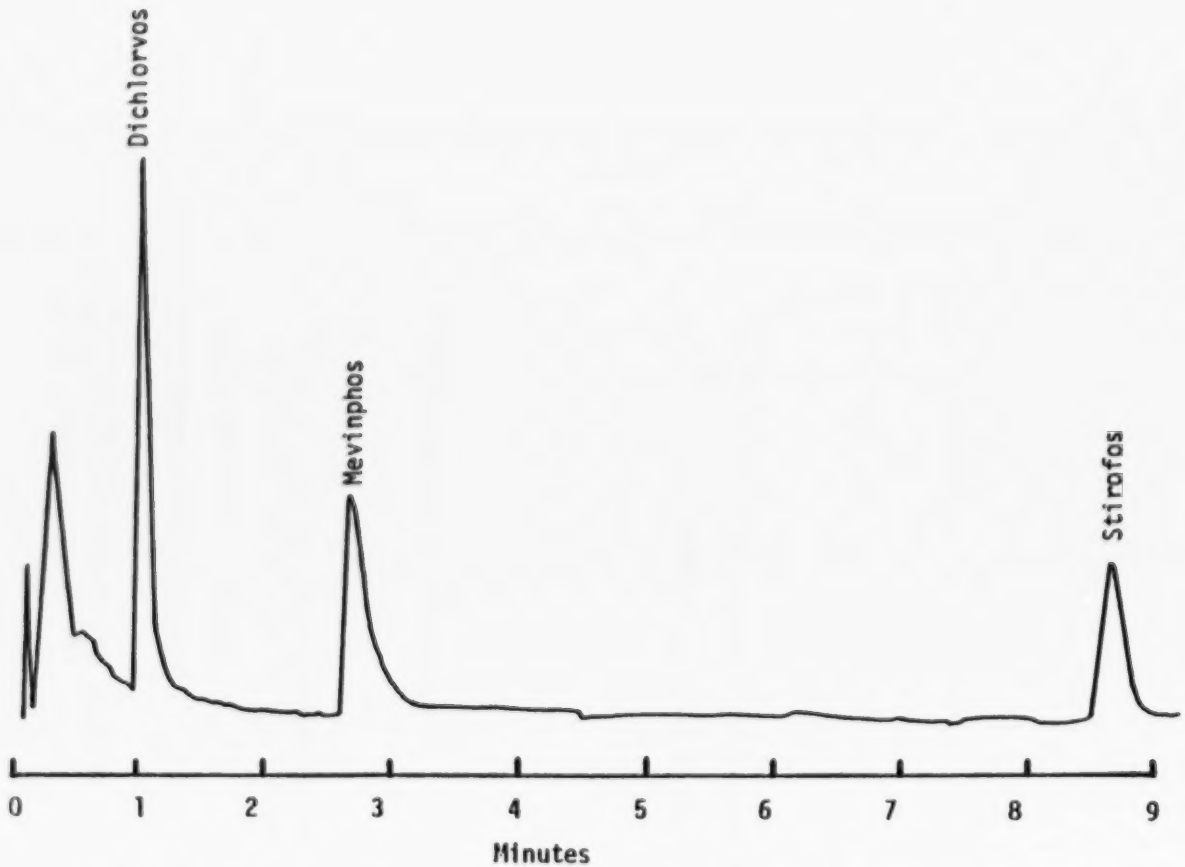

Figure 2. Gas chromatogram of organophosphorus pesticides on Column 1b. For conditions, see Table 1.

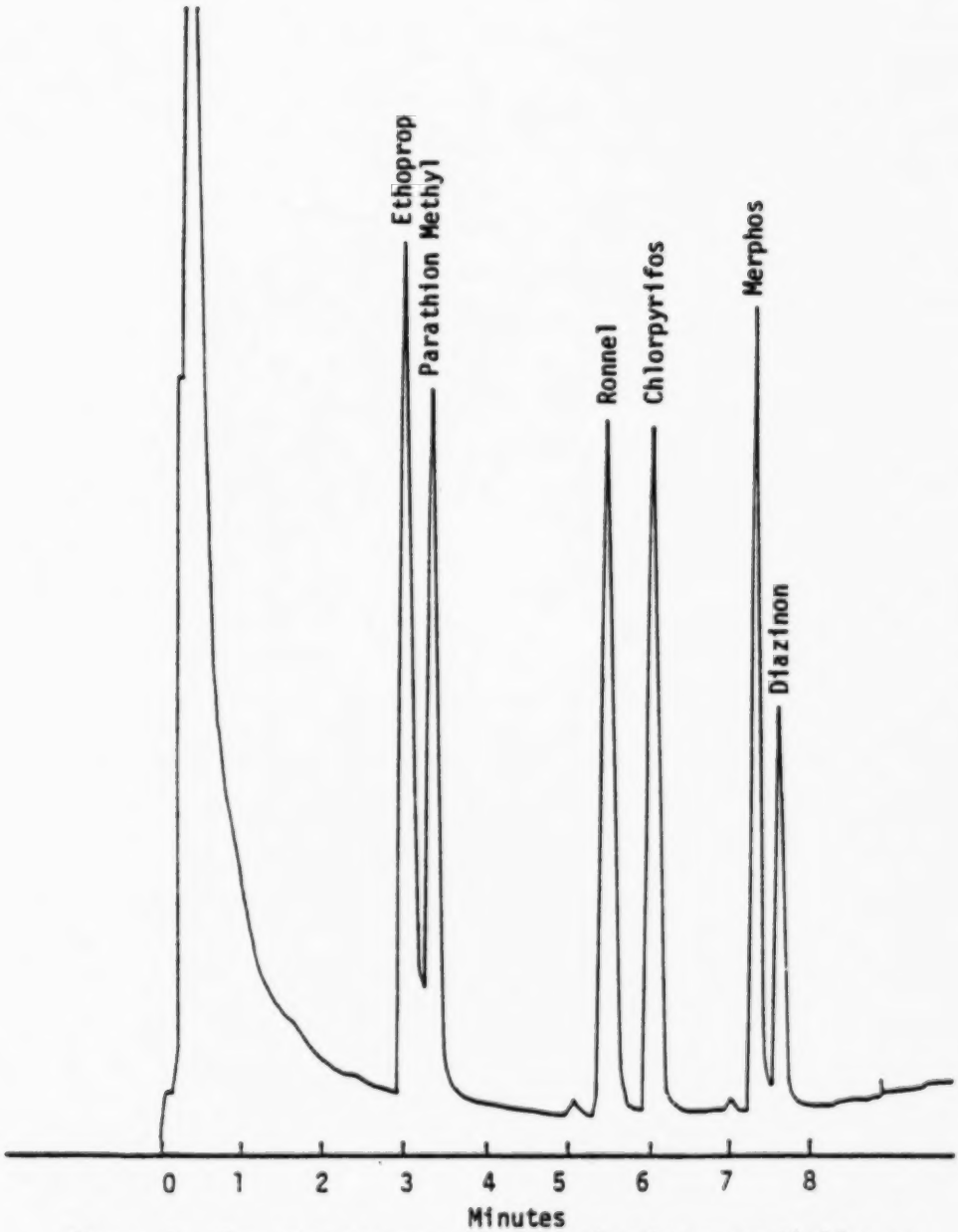

Figure 3. Gas chromatogram of organophosphorus pesticides on Column 2. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF PENTACHLOROPHENOL SALT IN WASTEWATER

METHOD 625

1. Scope and Application

1.1 This method covers the determination of a number of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography. The parameters listed in Tables 1 and 2 may be qualitatively and quantitatively determined using this method.

1.2 The method may be extended to include the parameters listed in Table 3. Benzidine can be subject to oxidative losses during solvent concentration. α-BHC, γ-BHC, endosulfan I and II, and endrin are subject to decomposition under the alkaline conditions of the extraction step. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution and photochemical decomposition. N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. The preferred method for each of these parameters is listed in Table 3.

1.3 This is a gas chromatography/mass spectrometry (GC/MS) method applicable to the determination of the compounds listed in Tables 1, 2, and 3 in municipal and industrial discharges as provided under 40 CFR 136.1. Until the U.S. Environmental Protection Agency establishes performance criteria based upon the results of interlaboratory testing, any alternative GC/MS method which meets the performance criteria described in Section 8.2 will be permitted. Performance must be verified for such modification by analyzing wastewater as described in Section 8.2.2. In addition, the laboratory must successfully participate in the applicable performance evaluation studies.

1.4 The method detection limit (MDL, defined in Section 16)[1] for each parameter is listed in Tables 4 and 5. The MDL for a specific wastewater differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is serially extracted with methylene chloride at a pH greater than 11 and again at pH less than 2 using a separatory funnel or a continuous extractor. The methylene chloride extract is dried and concentrated to a volume of 1 mL. Chromatographic conditions are described which permit the separation and measurement of the parameters in the extract. Qualitative identification is performed using the retention time and the relative abundance of three characteristic ions. Quantitative analysis is performed using either

external or internal standard techniques with a single characteristic ion.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained dry, and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store it inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled.

3.3 The base-neutral extraction may cause significantly reduced recovery of phenol, 2-methylphenol, and 2,4-dimethylphenol. The analyst must recognize that results obtained under these conditions are minimum concentrations.

3.4 The packed gas chromatographic columns recommended for the basic fraction may not exhibit sufficient resolution for certain isomeric pairs. These include anthracene and phenanthrene; chrysene and benzo(a)anthracene; and benzo(b)fluoranthene and benzo(k)fluoranthene. The gas chromatograph retention time and mass spectra are not sufficiently different to make an unambiguous distinction between these compounds. Alternative techniques should be used to identify and quantify these specific compounds. See method 610.

3.5 In samples that contain an inordinate number of interferences, the use of chemical ionization (CI) mass spectrometry may make identification easier. Tables 6 and 7 give characteristic CI ions for most of the compounds covered by this method. The use of CI mass spectrometry to support electron ionization (EI) mass spectrometry is encouraged but not required.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst[4-6].

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens; benzo(a)anthracene, benzidine, 3,3'-dichlorobenzidine, benzo(a)pyrene, α-BHC, β-BHC, δ-BHC, γ-BHC, dibenzo(a,h) anthracene, N-nitrosodimethylamine, 4,4'-DDT and polychlorinated biphenyls.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL, with Teflon stopcock.

5.2.2 Drying column—19 mm ID chromatographic column with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish—Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish—Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.2.8 Continuous liquid-liquid extractors—Equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication. (Hershberg-Wolf Extractor-Ace Glass Company, Vineland, N.J. P/N 6841-10 or equivalent.)

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (±2 °C). The bath should be used in a hood.

5.5 Balance—Analytical, capable of accurately weighing 0.0001 g.

5.6 GC/MS system.

5.6.1 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. The injection port must be designed for on-column injection when using packed columns and for splitless injection when using capillary columns.

5.6.2 Column for Base Neutrals—1.8 m long × 2 mm ID glass, packed with Supelcoport (100/120 mesh) coated with 3% SP-2250 or equivalent. This column was used to develop the accuracy and precision statements in Table 6 and the MDL data in Table 4. Guidelines for the use of alternate column packings are provided in Section 13.1.

5.6.3 Column for Acids—1.8 m long × 2 mm ID glass, packed with Supelcoport (100/120 mesh) coated with 1% SP-1240 DA or equivalent. This column was used to develop the accuracy and precision statements in Table 7, and the MDL data in Table 5. Guidelines for the use of alternate column packings are given in Section 13.1.

5.6.4 Mass Spectrometer—Capable of scanning from 35 to 450 amu every seven seconds or less utilizing a 70 volt (nominal) electron energy in the electron impact ionization mode and producing a mass spectrum which meets all the criteria in Table 9 when 50 ng of decafluorotriphenyl phosphine (DFTPP; bis(perfluorophenyl) phenyl phosphine) is injected through the gas chromatographic inlet. Any gas chromatograph to mass spectrometer interface that gives acceptable calibration points at 50 ng per injection for each compound of interest in Tables 1 through 3 and achieves all acceptable performance criteria (Section 12) may be used. Gas chromatograph to mass spectrometer interfaces constructed of all glass or glass lined materials are recommended. Glass can be deactivated by silanizing with dichlorodimethylsilane.

5.6.5 A computer system must be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer must have software that allows searching any GC/MS data file for ions of a specific mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundance in any EICP between specified time or scan number limits.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Sodium hydroxide solution (10 N)—Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium thiosulfate—(ACS) Granular.

6.4 Sulfuric acid solution (1 + 1)—Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.5 Acetone, methanol, methylene chloride—Pesticide quality or equivalent.

6.6 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400 °C for four hours in a shallow tray.

6.7 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is assayed at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store in at 4 °C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check samples, that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.7.3 Stock standard solutions must be replaced after six months or sooner if comparison with quality control check samples indicate a problem.

6.8 Surrogate standard spiking solutions—select a minimum of three surrogate compounds from Table 8. Prepare a surrogate standard spiking solution at a concentration of 100 µg/1.00 ML in acetone. Addition of 1.00 mL of this solution to 1000-mL of sample is equivalent to a concentration of 100 µg/L of each surrogate standard. Store the spiking solutions at 4 °C in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check samples indicate a problem. Surrogate standard spiking solutions, appropriate for use with this method will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.9 DFTPP standard—Prepare a 25 ng/µL solution of DFTPP in acetone.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Tables 4 or 5. The GC/MS system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetone. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system.

7.2.2 Analyze 2 to 5 µL of each calibration standard and tabulate the area responses of the primary characteristic ion of each standard (Tables 4 and 5) against the mass injected. The results may be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over

the working range (<10% relative standard deviation, RSD), linearity through the origin may be assumed and the average ratio or calibration factor may be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Table 8 lists some recommended internal standards. Phenanthrene-d_{10} has been used for this purpose. Use the base peak ion as the primary ion for quantification of the standards. If interferences are noted, use the next two most intense ions as the secondary ions.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding appropriate volumes of one or more stock standards to a volumetric flask. To each calibration standard or standard mixture, add a known constant amount of one or more internal standards, and dilute to volume with acetone. One of the calibration standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system.

7.3.2 Analyze 2 to 5 µL of each calibration standard and tabulate the area of the primary characteristic ion (Tables 4 and 5) against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Area of the characteristic ion for the parameter to be measured.

A_{is} = Area of the characteristic ion for the internal standard.

C_{is} = Concentration of the internal standard, (µg/L).

C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike all samples with surrogate standards to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each parameter to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate and 1.0 mL of the surrogate standard dosing solution (Section 6.8) to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for all parameters and surrogate standards. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 6 or 7, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > p$ or $|X - R| > p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the result of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria for each of the surrogate standards.

8.3.1 Calculate upper and lower control limits for method performance for each surrogate standard, using the values for R and s calculated in Section 8.2.3:

$$\text{Upper Control Limit (UCL)} = R + 3s$$
$$\text{Lower Control Limit (LCL)} = R - 3s$$

The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become avail-

able from the U.S. Environmental Protection Agency.

8.3.2 For each surrogate standard, the laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[7]

8.4 The laboratory is required to spike all samples with the surrogate standard spiking solution to monitor spike recoveries. If the recovery for any surrogate standard does not fall within the control limits for method performance, the results reported for that sample must be qualified as described in Section 15.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C from the time of collection until extraction. Fill the sample bottles and, if residual chlorine is present, add 80 mg of sodium thiosulfate per each liter of water. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used to measure the residual chlorine[9]. Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. Separatory Funnel Extraction

10.1 Samples are usually extracted using separatory funnel techniques. If emulsions will prevent achieving acceptable solvent recovery with separatory funnel extractions, continuous extraction (Section 11) may be used. The separatory funnel extraction scheme described below assumes a sample volume of one-liter. When sample volumes of two liters are to be extracted, use 250-, 100-, and 100-mL volumes of methylene chloride for the serial extraction of the base/neutrals and 200-, 100-, and 100-mL volumes of methylene chloride for the acids.

10.2 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Pipet 1.00 mL surrogate standard spiking solution into the separatory funnel and mix well. Check the pH of the sample with wide-range pH paper and adjust to pH > 11 with 10 N sodium hydroxide.

10.3 Add 60 mL methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-ml Erlenmeyer flask. If the emulsion cannot be broken (recovery of less than 80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and proceed as described in Section 11.3.

10.4 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Label the combined extract as base/neutral fraction.

10.5 Adjust the pH of the aqueous phase to less than 2 using sulfuric acid (1 + 1). Serially extract three times with 60-mL aliquots of methylene chloride. Collect and combine the extracts in a 250-mL Erlenmeyer flask and label the combined extract as the acid fraction.

10.6 For each fraction, assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.7 For each fraction, pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.8 To the evaporative flask for each fraction, add one or two clean boiling chips and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (60 ° to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL remove the K-D apparatus from the water bath and

allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation.

10.9 Add another one or two clean boiling chips to the concentrator tube and attach a two-ball micro Snyder column. Prewet the Snyder column by adding about 0.5 mL of methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (60° to 65 °C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain for at least 10 minutes while cooling. Remove the Snyder column and rinse its the flask and its lower joint into the concentrator tube with 0.2 mL of acetone or methylene chloride. Adjust the final volume to 1.0 mL with the solvent. Stopper the concentrator tube and store refrigerated if GC/MS analysis will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles and labeled base/neutral or acid fraction as appropriate.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Continuous Extraction

11.1 When experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered in Section 10.3, using a separatory funnel, a continuous extractor should be used.

11.2 Mark the water meniscus on the side of the sample bottle for later measurement of the sample volume. Check the pH of the sample with wide-range pH paper and adjust to pH 11 with 10 N sodium hydroxide. Transfer the sample to the continuous extractor and using a pipet, add 1.00 mL of surrogate standard spiking solution and mix well. Add 60 mL of methylene chloride to the sample bottle, seal and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the extractor.

11.3 Repeat the sample bottle rinse with an additional 50- to 100-mL portion of methylene chloride and add the rinse to the extractor.

11.4 Add 200 to 500 mL of methylene chloride to the distilling flask, add sufficient reagent water to ensure proper operation, and extract for 24 hours. Allow to cool, then detach the boiling flask, and dry, concentrate and seal the extract as in Section 10.6 through 10.9. Hold the aqueous phase for acid extraction (See Section 11.5).

11.5 Charge a clean distilling flask with 500 mL of methylene chloride and attach it to the continuous extractor. Carefully, adjust the pH of the aqueous phase to less than 2 using sulfuric acid (1 + 1). Extract for 24 hours. Dry, concentrate and label and seal the extract as described in Sections 10.6 through 10.9.

12. Daily GC/MS Performance Tests

12.1 At the beginning of each day that analyses are to be performed, the GC/MS system must be checked to see that acceptable performance criteria are achieved for DFTPP. Each day that benzidine is to be determined, the tailing factor criterion described in Section 12.4 must be achieved. Each day the acids are to be determined, the tailing factor criterion in Section 12.5 must be achieved.

12.2 These DFTPP performance test require the following instrumental parameters.

Electron Energy	70 volts (nominal)
Mass Range	35 to 450 amu
Scan Time	to give at least 5 scans per peak but not to exceed 7 seconds per scan.

12.3 DFTPP performance test[10]—At the beginning of each day, inject 2μL (50 ng) of DFTPP standard solution. Obtain a background corrected mass spectra of DFTPP and check that all the key ion criteria in Table 9 are achieved. If all the criteria are not achieved, the analyst must retune the mass spectrometer and repeat the test until all criteria are achieved. The performance criteria must be achieved before any samples, blanks, or standards are analyzed. The tailing factor tests in Section 12.4 and 12.5 may be performed simultaneously with the test.

12.4 Column performance test for base/neutrals—At the beginning of each day that the base-neutral fraction is to be analyzed for benzidine, the benzidine tailing factor must be calculated. Inject 100 ng of benzidine either separately or as a part of a standard mixture that may contain DFTPP and calculate the tailing factor. The benzidine tailing factor must be less than 3.0. Calculation of the tailing factor is illustrated in Figure 13.[11] Replace the column packing if the tailing factor criterion cannot be achieved.

12.5 Column performance for acids—At the beginning of each day that the acids are to be determined, inject 50 ng of pentachlorophenol either separately or as a part of a standard mix that may contain DFTPP. The tailing factor for pentachlorophenol must be less than five. Calculation of the tailing factor is illustrated in Figure 13[11]. Replace the column packing if the tailing factor criterion cannot be achieved.

13. Gas Chromatography/ Mass Spectrometry

13.1 Table 4 summarizes the recommended gas chromatographic operating conditions for the base/neutral fraction. Table 5 summarizes the recommended gas chromatographic operating conditions for determination of the acid fraction. These tables include retention times and MDL that were achieved under these conditions. Examples of the parameter separations achieved by these columns are shown in Figures 1 through 12. Other packed columns or chromatographic conditions may be used if the requirements of Section 8.2 and Section 12 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 and Section 12 are met.

13.2 After the GC/MS performance requirements of Section 12, calibrate the system daily as described in Section 7.

13.3 If the internal standard approach is being used, the internal standard must be added to sample extract and mixed thoroughly, immediately, before injection into the instrument. This minimizes losses due to adsorption, chemical reaction or evaporation.

13.4 Inject 2 to 5 μL of the sample extract using the solvent-flush

technique[12]. Smaller (1.0 µL) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL.

13.5 If the response for any ion exceeds the working range of the GC/MS system, dilute the extract and reanalyze.

13.6 Perform all qualitative and quantitative measurements as described in Sections 14 and 15. When the extracts are not being used for analyses, store them at 4 °C protected from light in screw-cap vials equipped with unpierced Teflon-lined septa.

14. Qualitative Identification

14.1 Obtain an EICP for the primary ion and the two other ions listed in Tables 4 and 5. See Section 7.3 for ions to be used with internal and surrogate standards. The following criteria must be met to make a qualitative identification.

14.1.1 The characteristic ions for each compound of interest must maximize in the same or within one scan of each other.

14.1.2 The retention time must fall within ±30 seconds of the retention time of the authentic compound.

14.1.3 The relative peak heights of the three characteristic ions in the EICP's must fall within ±20% of the relative intensities of these ions in a reference mass spectrum. The reference mass spectrum can be obtained by a standard analyzed in the GC/MS system or from a reference library.

14.2 Structural isomers that have very similar mass spectra and less than 30 seconds difference in retention time, can be explicitly identified only if the resolution between authentic isomers in a standard mix is acceptable. Acceptable resolution is achieved if the baseline to valley height between the isomers is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

15. Calculations

15.1 When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion in Tables 4 and 5. Use the base peak ion for internal and surrogate standards. If the sample produces an interference for the first listed ion, use a secondary ion to quantitate. Quantitation will be performed using external or internal standard techniques.

15.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the area of the characteristic ion using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

Eq. 2. $$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

- A = Amount of material injected, in nanograms.
- V_i = Volume of extract injected (µL).
- V_t = Volume of total extract (µL).
- V_s = Volume of water extracted (mL).

15.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

Eq. 3

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

- A_s = Area of the characteristic ion for the parameter to be measured.
- A_{is} = Area of the characteristic ion for the internal standard.
- I_s = Amount of internal standard added to each extract (µg).
- V_o = Volume of water extracted (liters).

15.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

15.3 If the surrogate standard recovery falls outside the control limits in Section 8.3, data for all parameters in that fraction of the sample must be labeled as suspect.

16. Method Performance

16.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the value is above zero[1]. The MDL concentrations listed in Tables 4 and 5 were obtained using reagent water[13].

16.2 The average recoveries and the average standard deviations of the percent recoveries, presented in Table 5, were the result of a study of the accuracy and precision of this method by several laboratories. The values listed represent the results from two to four laboratories[14].

16.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

17. Screening Procedure for 2,3,7,8-TCDD

17.1 If the sample must be screened for the presence of 2,3,7,8-TCDD, it is recommended that the reference material not be handled in the laboratory unless extensive safety precautions are employed. It is sufficient to analyze the base/neutral extract by selected ion monitoring (SIM) GC/MS techniques, as follows:

17.1.1 Concentrate the base/neutral extract to a final volume of 0.2 mL.

17.1.2 Adjust the temperature of the base/neutral column (Section 5.6.2) to 220 °C.

17.1.3 Operate the mass spectrometer to acquire data in the SIM mode using the ions at m/e 257, 320 and 322 and a dwell time no greater than 333 milliseconds per ion.

17.1.4 Inject 5 to 7 µL of the base/neutral extract. Collect SIM data for a total of 10 minutes.

17.1.5 The possible presence of 2,3,7,8-TCDD is indicated if all three ions exhibit simultaneous peaks at any point in the selected ion current profiles.

17.1.6 For each occurrence where the possible presence of 2,3,7,8-TCDD is indicated, calculate and retain the relative abundances of each of the three ions.

17.2 False positives to this test may be caused by the presence of single or coeluting combinations of compounds whose mass spectra contain all of these ions.

17.3 Conclusive results of the presence and concentration level of 2,3,7,8-TCDD can be obtained only from a properly equipped laboratory through the use of method 613 of other approved alternate test procedures.

References

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Table 1. Base/Neutral Extractables

Parameter	STORET No.	CAS No.
Acenaphthene	34205	83-32-9
Acenaphthylene	34200	208-96-8
Anthracene	34220	120-12-7
Aldrin	39330	309-00-2
Benzo(a)anthracene	34526	56-55-3
Benzo(b)fluoranthene	34230	205-99-2
Benzo(k)fluoranthene	34242	207-08-9
Benzo(a)pyrene	34247	50-32-8
Benzo(ghi)perylene	34521	191-24-2
Benzyl butyl phthalate	34292	85-68-7
β-BHC	39338	319-85-7
δ-BHC	34259	319-86-8
Bis(2-chloroethyl)ether	34273	111-44-4
Bis(2-chloroethoxy)methane	34278	111-91-1
Bis(2-ethylhexyl)phthalate	39100	117-81-7
Bis(2-chloroisopropyl)ether	34283	108-60-1
4-Bromophenyl phenyl ether	34636	101-55-3
Chlordane	39350	57-74-9
2-Chloronaphthalene	34581	91-58-7
4-Chlorophenyl phenyl ether	34641	7005-72-3
Chrysene	34320	218-01-9
4,4'-DDD	39310	72-54-8
4,4'-DDE	39320	72-55-9
4,4'-DDT	39300	50-29-3
Dibenzo(a,h)anthracene	34556	53-70-3
Di-n-butylphthalate	39110	84-74-2
1,3-Dichlorobenzene	34566	541-73-1
1,2-Dichlorobenzene	34536	95-50-1
1,4-Dichlorobenzene	34571	106-46-7
3,3'-Dichlorobenzidine	34631	91-94-1
Dieldrin	39380	60-57-1
Diethyl phthalate	34336	84-66-2
Dimethyl phthalate	34341	131-11-3
2,4-Dinitrotoluene	34611	121-14-2
2,6-Dinitrotoluene	34626	606-20-2
Di-n-octylphthalate	34596	117-84-0
Endosulfan sulfate	34351	1031-07-8
Endrin aldehyde	34366	7421-93-4
Fluoranthene	34376	206-44-0
Fluorene	34381	86-73-7
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Hexachlorobenzene	39700	118-74-1
Hexachlorobutadiene	34391	87-68-3
Hexachloroethane	34396	67-72-1
Indeno(1,2,3-cd)pyrene	34403	193-39-5
Isophorone	34408	78-59-1
Naphthalene	34696	91-20-3
Nitrobenzene	34447	98-95-3
N-Nitrosodi-n-propylamine	34428	621-64-7
PCB-1016	34671	12674-11-2
PCB-1221	39488	11104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5
Phenanthrene	34461	85-01-8
Pyrene	34469	129-00-0
Toxaphene	39400	8001-35-2
1,2,4-Trichlorobenzene	34551	120-82-1

Table 2. Acid Extractables

Parameter	STORET No.	CAS No.
4-Chloro-3-methylphenol	34452	59-50-7
2-Chlorophenol	34586	95-57-8
2,4-Dichlorophenol	34601	120-83-2
2,4-Dimethylphenol	34606	105-67-9
2,4-Dinitrophenol	34616	51-28-5
2-Methyl-4,6-dinitrophenol	34657	534-52-1
2-Nitrophenol	34591	88-75-5
4-Nitrophenol	34646	100-02-7
Pentachlorophenol	39032	87-86-5
Phenol	34694	108-95-2
2,4,6-Trichlorophenol	34621	88-06-2
Pentachlorophenol Salt	—	131-52-2

Table 3. Additional Extractable Parameters[a]

Parameter	STORET No.	CAS No.	Method
Benzidine	39120	92-87-5	605
α-BHC	39337	319-84-6	608
γ-BHC	39340	58-89-8	608
Endosulfan I	34361	959-98-8	608
Endosulfan II	34356	33213-65-9	608
Endrin	39390	72-20-8	608
Hexachlorocyclopentadiene	34386	77-47-4	612
N-Nitrosodimethylamine	34438	62-75-9	605
N-Nitrosodiphenylamine	34433	86-30-6	605

[a]See Section 1.2 of method

Table 4. Chromatographic Conditions, Method Detection Limits and Characteristic Ions for Base/Neutral Extractables

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)	Characteristic Ions: Electron Impact, Primary	Electron Impact, Secondary		Chemical Ionization (Methane)		
1,3-Dichlorobenzene	7.4	1.9	146	148	113	146	148	150
1,4-Dichlorobenzene	7.8	4.4	146	148	113	146	148	150
Hexachloroethane	8.4	1.6	117	201	199	199	201	203
Bis(2-chloroethyl)ether	8.4	5.7	93	63	95	63	107	109
1,2-Dichlorobenzene	8.4	1.9	146	148	113	146	148	150
Bis(2-chloroisopropyl)ether	9.3	5.7	45	77	79	77	135	137
N-Nitrosodi-n-propyl amine			130	42	101			
Nitrobenzene	11.1	1.9	77	123	65	124	152	164
Hexachlorobutadiene	11.4	0.9	225	223	227	223	225	227
1,2,4-Trichlorobenzene	11.6	1.9	180	182	145	181	183	209
Isophorone	11.9	2.2	82	95	138	139	167	178
Naphthalene	12.1	1.6	128	129	127	129	157	169
Bis(2-chloroethoxy)methane	12.2	5.3	93	95	123	65	107	137
Hexachlorocyclopentadiene[a]	13.9		237	235	272	235	237	239
2-Chloronaphthalene	15.9	1.9	162	164	127	163	191	203
Acenaphthylene	17.4	3.5	152	151	153	152	153	181
Acenaphthene	17.8	1.9	154	153	152	154	155	183
Dimethyl phthalate	18.3	1.6	163	194	164	151	163	164
2,6-Dinitrotoluene	18.7	1.9	165	89	121	183	211	223
Fluorene	19.5	1.9	166	165	167	166	167	195
4-Chlorophenyl phenyl ether	19.5	4.2	204	206	141			
2,4-Dinitrotoluene	19.8	5.7	165	63	182	183	211	223
Diethylphthalate	20.1	22	149	177	150	177	223	251
N-Nitrosodiphenylamine[a]	20.5	1.9	169	168	167	169	170	198
Hexachlorobenzene	21.0	1.9	284	142	249	284	286	288
α-BHC[a]	21.1		183	181	109			
4-Bromophenyl phenyl ether	21.2	1.9	248	250	141	249	251	277
γ-BHC[a]	22.4		183	181	109			
Phenanthrene	22.8	5.4	178	179	176	178	179	207
Anthracene	22.8	1.9	178	179	176	178	179	207
β-BHC	23.4	4.2	181	183	109			
Heptachlor	23.4	1.9	100	272	274			
δ-BHC	23.7	3.1	183	109	181			
Aldrin	24.0	1.9	66	263	220			

Table 4. *(Continued)*

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)	Characteristic Ions: Electron Impact, Primary	Electron Impact, Secondary		Chemical Ionization (Methane)		
Dibutyl phthalate	24.7	2.5	149	150	104	149	205	279
Heptachlor epoxide	25.6	2.2	353	355	351			
Endosulfan I*	26.4	–	237	339	341			
Fluoranthene	26.5	2.2	202	101	100	203	231	243
Dieldrin	27.2	2.5	79	263	279			
4,4'-DDE	27.2	5.6	246	248	176			
Pyrene	27.3	1.9	202	101	100	203	231	243
Endrin*	27.9	–	81	263	82			
Endosulfan II*	28.6	–	237	339	341			
4,4'-DDD	28.6	2.8	235	237	165			
Benzidine*	28.8	44	184	92	185	185	213	225
4,4'-DDT	29.3	4.7	235	237	165			
Endosulfan sulfate	29.8	5.6	272	387	422			
Endrin aldehyde	–	–	67	345	250			
Butyl benzyl phthalate	29.9	2.5	149	91	206	149	299	327
Bis(2-ethylhexyl) phthalate	30.6	2.5	149	167	279	149		
Chrysene	31.5	2.5	228	226	229	228	229	257
Benzo(a)anthracene	31.5	7.8	228	229	226	228	229	257
3,3'-Dichlorobenzidine	32.2	16.5	252	254	126			
Di-n-octylphthalate	32.5	2.5	149					
Benzo(b)fluoranthene	34.9	4.8	252	253	125	252	253	281
Benzo(k)fluoranthene	34.9	2.5	252	253	125	252	253	281
Benzo(a)pyrene	36.4	2.5	252	253	125	252	253	281
Indeno(1,2,3-c,d)pyrene	42.7	3.7	276	138	277	276	277	305
Dibenzo(a,h)anthracene	43.2	2.5	278	139	279	278	279	307
Benzo(ghi)perylene	45.1	4.1	276	138	277	276	277	305
N-Nitrosodimethyl amine*	–	–	42	74	44			
Chlordane**	19 to 30	–	373	375	377			
Toxaphene**	25 to 34	–	159	231	233			
PCB 1016**	18 to 30	–	224	260	294			
PCB 1221**	15 to 30	30	190	224	260			
PCB 1232**	15 to 32	–	190	224	260			
PCB 1242**	15 to 32	–	224	260	294			
PCB 1248**	12 to 34	–	294	330	362			
PCB 1254**	22 to 34	36	294	330	362			
PCB 1260**	23 to 32	–	330	362	394			

*See Section 1.2.

**These compounds are mixtures of various isomers. (See Figures 2 to 12)

Gas Chromatographic conditions: Glass column 1.8 m long × 2 mm ID packed with Supelcoport (100/120 mesh) coated with 3% SP-2250. Carrier gas: helium at a flow rate of 30 mL per min.
Temperature: Isothermal at 50°C for 4 min., then 8° per min to 270°C. Hold at 270°C for 30 min.

Table 5. Chromatographic Conditions, Method Detection Limits and Characteristic Ions for Acid Extractables

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)	Characteristic Ions: Electron Impact, Primary	Electron Impact, Secondary		Chemical Ionization (Methane)		
2-Chlorophenol	5.9	3.3	128	64	130	129	131	157
2-Nitrophenol	6.5	3.6	139	65	109	140	168	122
Phenol	8.0	1.5	94	65	66	95	123	135
2,4-Dimethylphenol	9.4	2.7	122	107	121	123	151	163
2,4-Dichlorophenol	9.8	2.7	162	164	98	163	165	167
2,4,6-Trichlorophenol	11.8	2.7	196	198	200	197	199	201
4-Chloro-3-methylphenol	13.2	3.0	142	107	144	143	171	183
2,4-Dinitrophenol	15.9	42	184	63	154	185	213	225
2-Methyl-4,6-dinitrophenol	16.2	24	198	182	77	199	227	239
Pentachlorophenol	17.5	3.6	266	264	268	267	265	269
4-Nitrophenol	20.3	2.4	65	139	109	140	168	122

Chromatographic conditions: 1.8 m long × 2 mm ID glass column packed with Supelcoport (100/120 mesh) coated with 1% SP-1240. Carrier gas: helium at a flow rate of 30 mL per min. Column temperature, isothermal at 70°C for 2 min, then 8° per min, to 200°.

Table 6. Accuracy and Precision for Base/Neutral Extractables

Parameter	Reagent Water		Wastewater	
	Average Percent Recovery	Standard Deviation (%)	Average Percent Recovery	Standard Deviation (%)
Acenaphthene	77	23	83	29
Acenaphthylene	78	22	82	23
Aldrin	72	6	–	–
Anthracene	84	14	76	22
Benzo(a)anthracene	83	19	75	28
Benzo(b)fluoranthene	96	68	41	21
Benzo(k)fluoranthene	96	68	47	27
Benzo(ghi)perylene	80	45	68	40
Benzo(a)pyrene	90	22	43	21
Benzidine	87	61	63	55
Butyl benzyl phthalate	47	32	74	43
β-BHC	69	25	–	–
δ-BHC	56	18	–	–
Bis(2-chloroethoxy)methane	84	33	82	74
Bis(2-chloroethyl)ether	56	36	72	37
Bis(2-chloroisopropyl)ether	71	33	71	39
Bis (2-ethylhexyl) phthalate	129	50	82	63
4-Bromophenyl phenyl ether	80	17	75	20
2-Chloronaphthalene	73	24	79	27
4-Chlorophenyl phenyl ether	45	11	–	–
Chrysene	83	19	75	28
4,4'-DDD	80	9	–	–
4,4'-DDE	69	20	–	–
4,4'-DDT	63	15	–	–
Dibenzo(a,h)anthracene	82	39	70	40
Di-n-butyl phthalate	70	25	93	51
1,2-Dichlorobenzene	59	27	62	28
1,3-Dichlorobenzene	55	28	54	24
1,4-Dichlorobenzene	61	31	63	35
3,3'-Dichlorobenzidine	184	174	143	145
Diethylphthalate	42	28	48	28
Dimethyl phthalate	25	33	35	36
2,4-Dinitrotoluene	83	32	79	34
2,6-Dinitrotoluene	79	18	79	25
Di-n-octylphthalate	97	37	89	62
Endosulfan sulfate	79	29	–	–
Fluoranthene	89	19	80	26
Fluorene	77	16	80	20
Heptachlor	69	6	–	–
Heptachlor epoxide	82	7	–	–
Hexachlorobenzene	79	20	71	22
Hexachlorobutadiene	46	25	48	28
Hexachlorocyclopentadiene	27	25	12	12
Hexachloroethane	46	21	52	26
Indeno (1,2,3-cd) pyrene	65	37	81	43
Isophorone	75	33	77	42
Naphthalene	6	32	75	35
Nitrobenzene	72	31	82	54
N-Nitrosodi-n-propylamine	68	39	76	45
N-Nitrosodiphenylamine	84	24	86	31
PCB-1221	77	11	–	–
PCB-1254	80	13	–	–
Phenanthrene	84	14	76	22
Pyrene	86	15	80	23
1,2,4-Trichlorobenzene	64	16	69	26

Spiked between 5 to 2400 μg/L.

Table 7. *Accuracy and Precision for Acid Extractables*

Parameter	Reagent Water		Wastewater	
	Average Percent Recovery	Standard Deviation (%)	Average Percent Recovery	Standard Deviation (%)
4-Chloro-3-methylphenol	79	18	75	21
2-Chlorophenol	70	23	71	25
2,4-Dichlorophenol	74	24	80	21
2,4-Dimethylphenol	64	25	58	26
2,4-Dinitrophenol	78	21	108	56
2-Methyl-4,6-dinitrophenol	83	18	90	35
4-Nitrophenol	41	20	43	16
2-Nitrophenol	75	25	75	27
Pentachlorophenol	86	20	66	36
Phenol	36	14	36	21
2,4,6-Trichlorophenol	77	20	81	20

Spiked from 10 to 1500 µg/L.

Table 8. *Suggested Internal and Surrogate Standards*

Base/Neutral Fraction	Acid Fraction
Aniline-d_5	2-Fluorophenol
Anthracene-d_{10}	Pentafluorophenol
Benzo(a)anthracene-d_{12}	Phenol-d_5
4,4'-Dibromobiphenyl	2-Perfluoromethyl phenol
4,4'-Dibromooctafluorobiphenyl	
Decafluorobiphenyl	
2,2'-Difluorobiphenyl	
4-Fluoroaniline	
1-Fluoronaphthylene	
2-Fluoronaphthylene	
Naphthalene-d_8	
Nitrobenzene-d_5	
2,3,4,5,6-Pentafluorobiphenyl	
Phenanthrene-d_{10}	
Pyridine-d_5	

Table 9. *DFTPP Key Ions and Ion Abundance Criteria*

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	less than 2% of mass 69
70	less than 2% of mass 69
127	40-60% of mass 198
197	less than 1% of mass 198
198	base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	greater than 1% of mass 198
441	present but less than mass 443
442	greater than 40% of mass 198
443	17-23% of mass 442

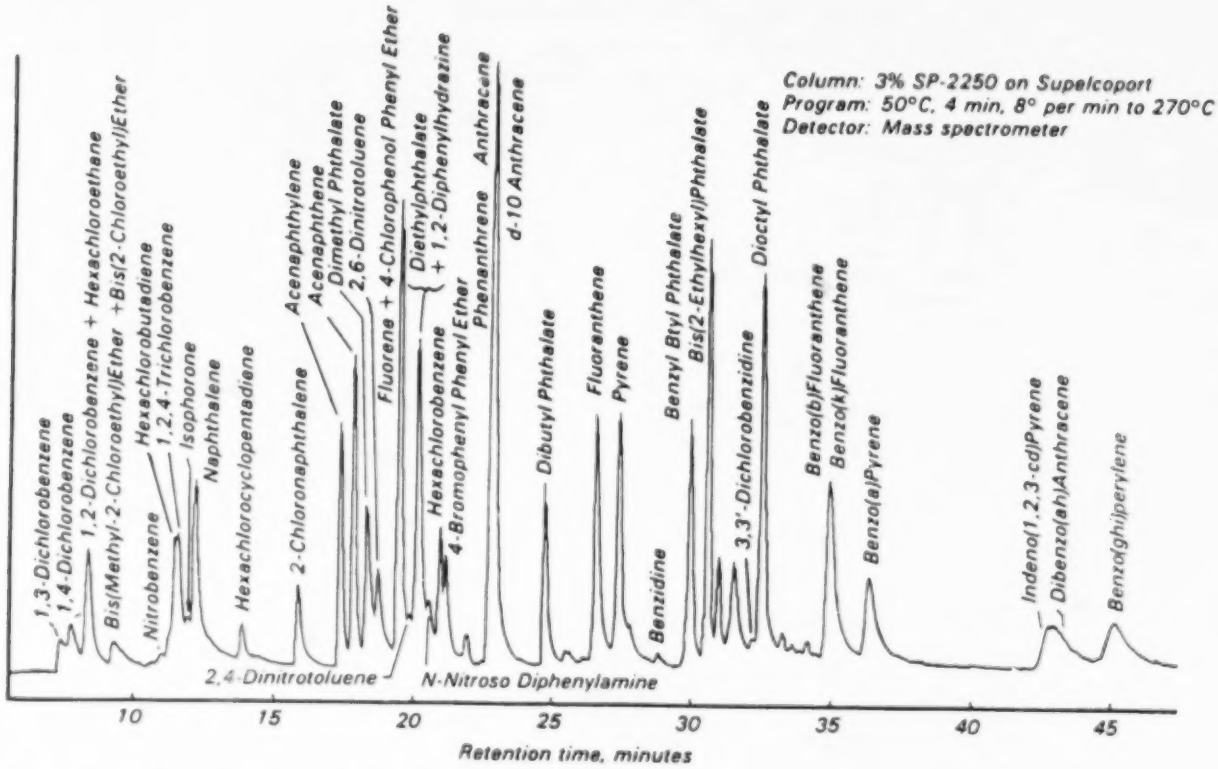


Figure 1. *Gas chroamatogram of base/neutral fraction.*

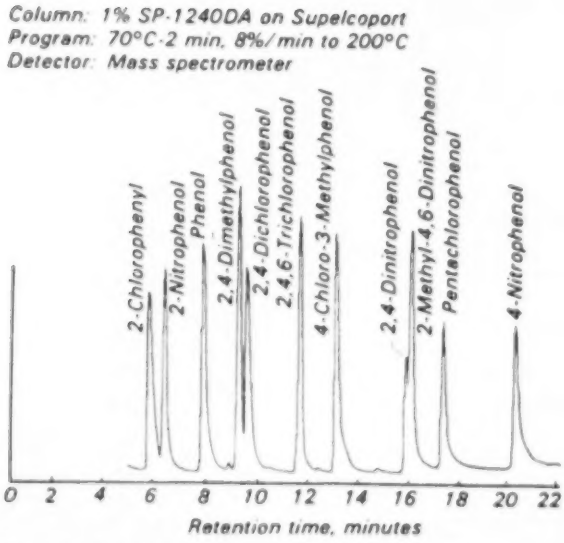


Figure 2. *Gas chromatogram of acid fraction.*

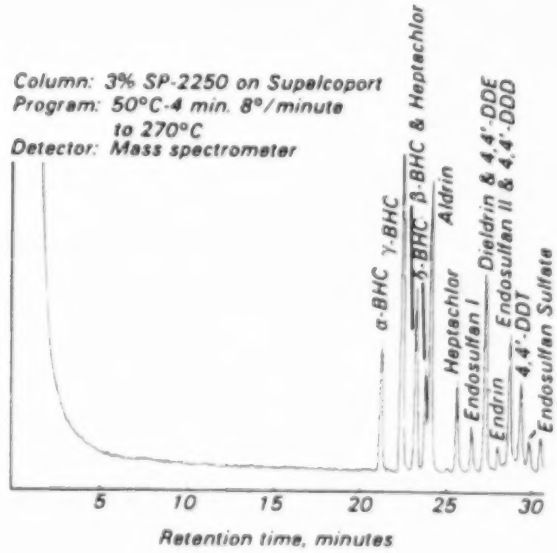


Figure 3. *Gas chromatogram of pesticide fraction.*

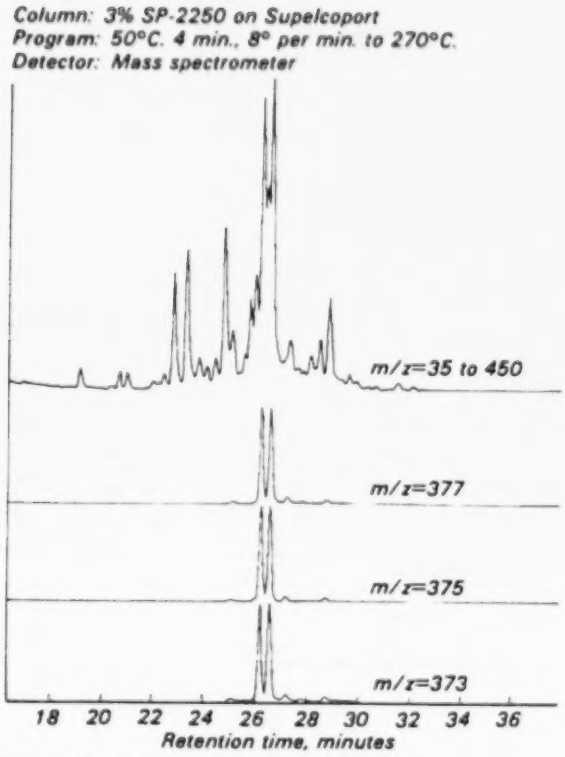


Figure 4. *Gas chromatogram of chlordane.*

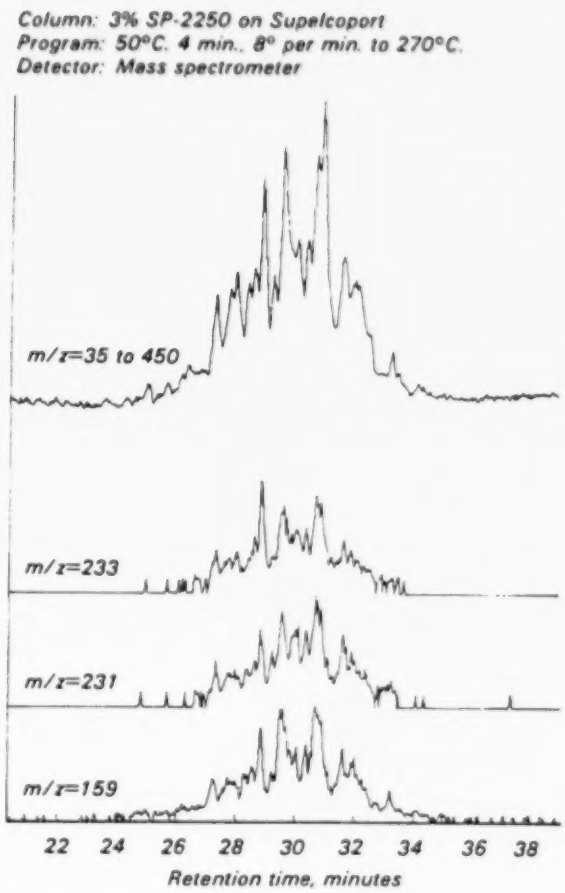


Figure 5. *Gas chromatogram of toxaphene.*

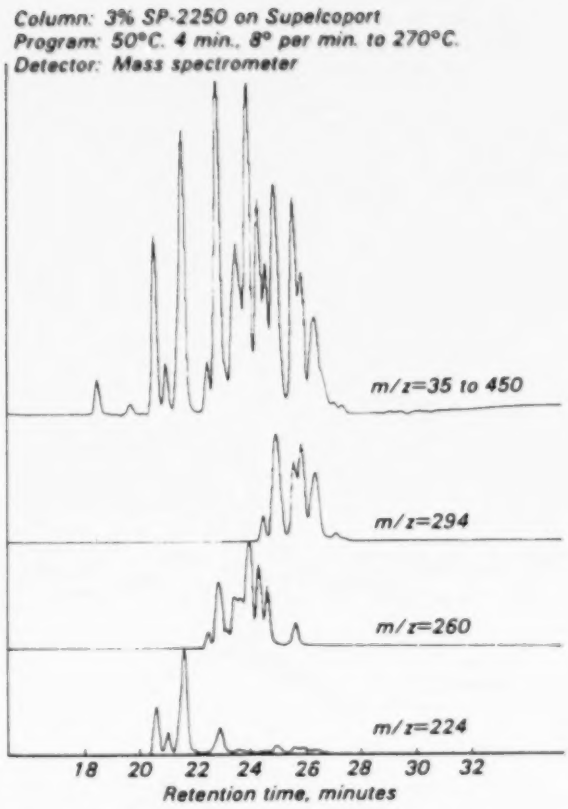


Figure 6. *Gas chromatogram of PCB-1016.*

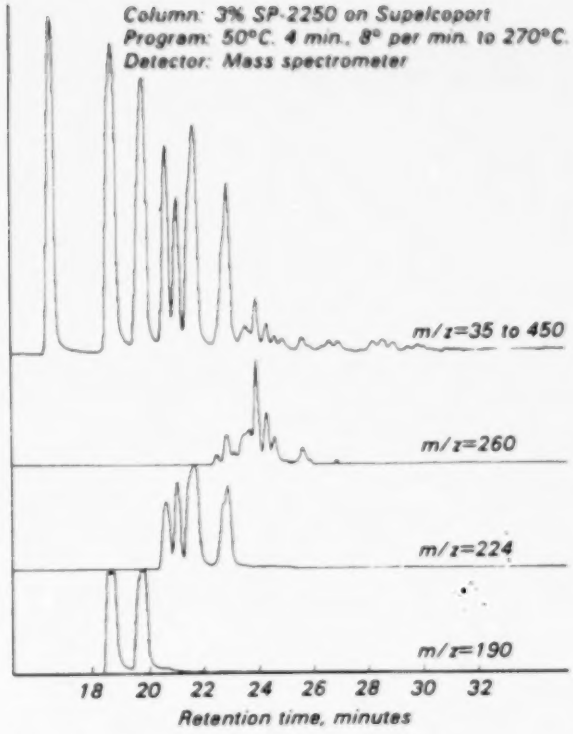


Figure 7. *Gas chromatogram of PCB-1221.*

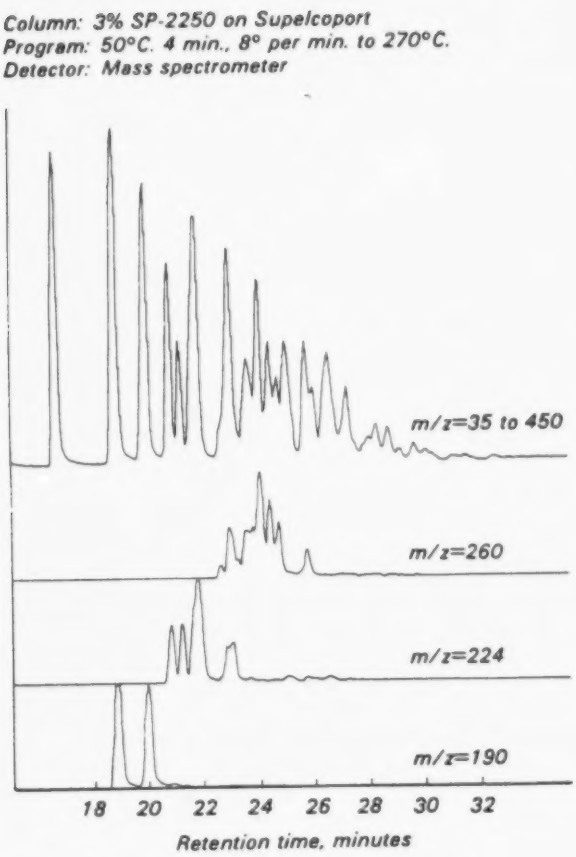


Figure 8. *Gas chromatogram of PCB-1232.*

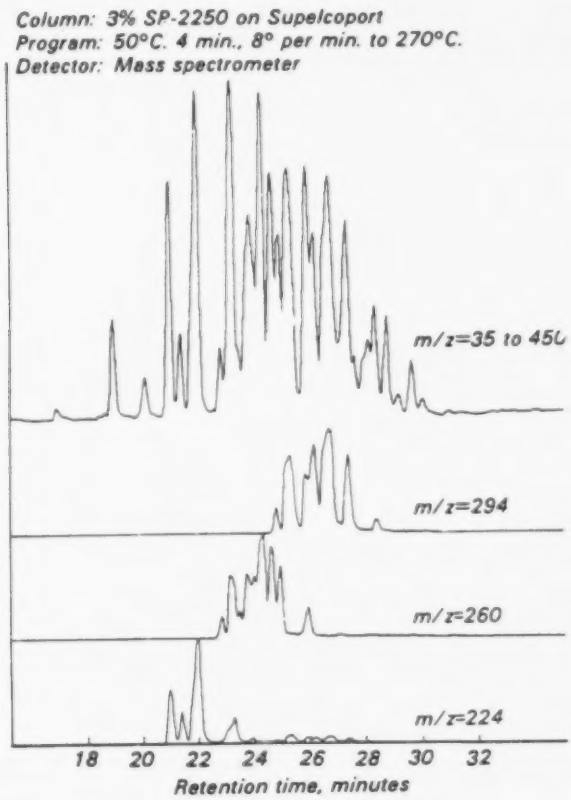


Figure 9. *Gas chromatogram of PCB-1242.*

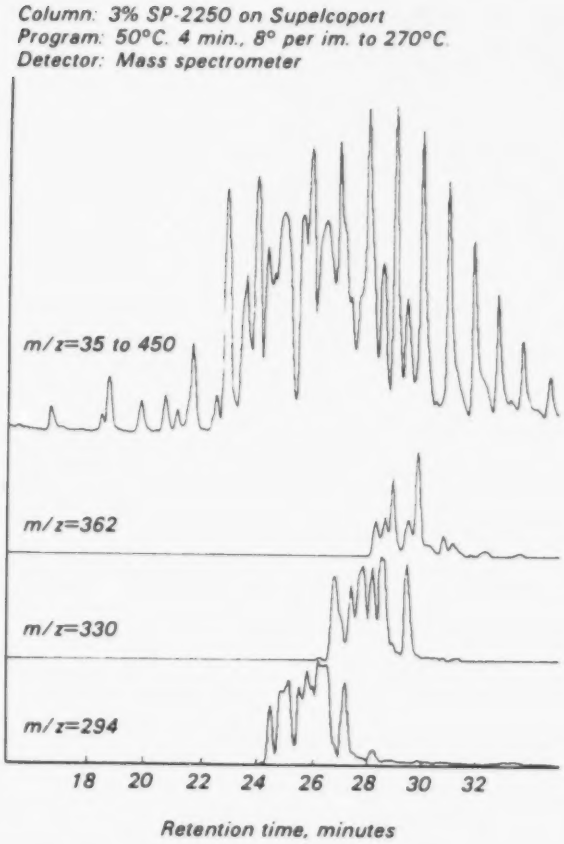


Figure 10. *Gas chromatogram of PCB-1248.*

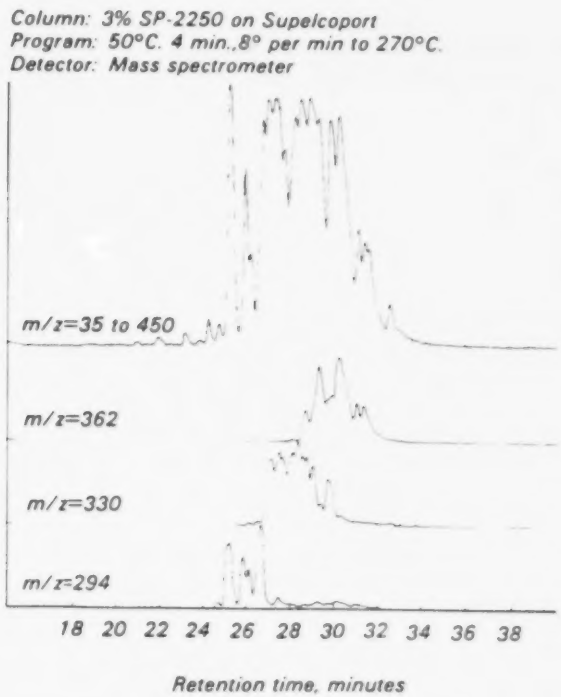


Figure 11. *Gas chromatogram of PCB-1254.*

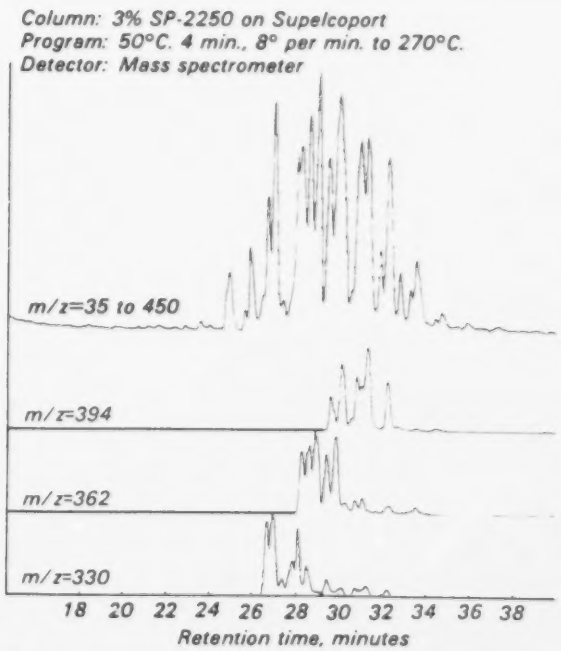
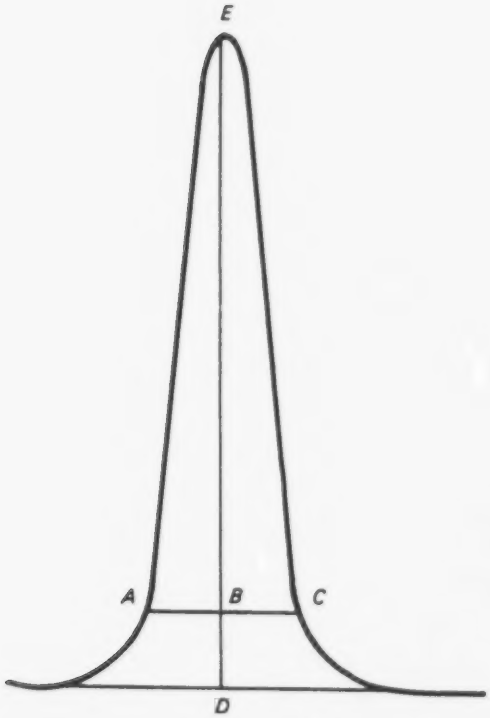


Figure 12. *Gs chromatogram of PCB-1260.*



$$\text{Tailing Factor} = \frac{BC}{AB}$$

Example calculation:

Peak Height = DE = 100mm
10% Peak Height = BD = 10 mm
Peak Width at 10% Peak Height = AC = 23 mm
AB = 11 mm
BC = 12 mm

Therefore: Tailing Factor $= \frac{12}{11} = 1.1$

Figure 13. *Tailing factor calculation.*

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BENFLURALIN, ETHALFLURALIN, ISOPROPALIN AND PROFLURALIN IN WASTEWATER

METHOD 627

1. Scope and Application

1.1 This method covers the determination of certain dinitroaniline pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Benfluralin	39002	1861-40-1
Ethalfluralin	---	55283-68-6
Isopropalin	---	33820-53-0
Profluralin	---	26399-36-0
Trifluralin	39030	1582-09-8

1.2 This method fails to distinguish between benfluralin, ethalfluralin and trifluralin. When more than one of these materials may be present in a sample, the results are reported as trifluralin.

1.3 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.4 The method detection limits (MDL, defined in Section 15) for four of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as several others in the 600-method series. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under Gas Chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.7 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column within the limitations described in Section 1.2. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture (EC) gas chromatography.[1]

2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large late eluting peaks. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.[3,4]

3.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require special cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL.

Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 1.5% OV-17/1.95% OV-210 on Gas Chrom Q (100/120 mesh) or

equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with Ultrabond 20M (100/120 mesh) or equivalent.

5.6.3 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, methylene chloride - Pesticide quality or equivalent.

6.3 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.4 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.4.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality hexane and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.4.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.4.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. These parameters do not adequately resolve benfluralin, ethalfluralin and trifluralin. When more than one of these compounds may be present in a sample, the instrument must be calibrated with trifluralin. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these

limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and

the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Table 2 provides single operator recovery and precision for isopropalin, profluralin and trifluralin. Similar results should be expected for benfluralin and ethalfluralin. Compare these results to the values calculated in Section 8.2.3.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[8] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[8]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[9] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding

about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if

the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7. Since the gas chromatographic conditions provided do not adequately separate benfluralin, ethalfluralin and trifluralin, calibrate with trifluralin if more than one of these materials may be present in a sample.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[10] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results. Results for benfluralin and ethalfluralin must be reported as trifluralin unless the sample has been characterized beyond the capabilities provided in this method.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[11]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[12]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[13] The MDL concentrations listed in Table 1 were obtained using reagent water.[1]

15.2 In a single laboratory (West Cost Technical Services, Inc.), using reagent water and effluents from pesticide manufacturers and the average recoveries presented in Table 2 were obtained[1]. The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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TABLE 1

GAS CHROMATOGRAPHY AND METHOD
DETECTION LIMITS OF DINITROANILINES

Parameter	Retention Time (min) Column 1	Column 2	Method Detection Limit (µg/L)
Trifluralin	1.6	2.2	0.03
Benfluralin	1.6	2.3	ND
Ethalfluralin	1.6	2.3	ND
Profluralin	2.3	3.4	0.14
Isopropalin	6.4	6.3	0.02

ND = Not determined.

Column 1 conditions: Gas Chrom Q (100/200 mesh) coated with 1.5% OV-17/1.95% OV-210 packed in a 1.8 m long x 2 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 30 mL/min. Column temperature: isothermal at 190°C.

Column 2 conditions: Ultrabond 20M (100/120 mesh) packed in a 1.8 m long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature: held at 160°C for 2 min, then programmed to 200°C at 10°C/min.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Sample Type	Spike Range (µg/L)	Number of Replicates	Average Percent Recovery	Standard Deviation %
Benfluralin	IW	2.0	2	93	--
Isopropalin	DW	0.5	7	93	1.1
	IW	2.2	7	88	13.2
Profluralin	DW	0.5	7	99	9.0
	IW	2.04	7	73	5.8
Trifluralin	DW	0.5	7	97	1.8
	IW	2.08	7	77	20.0

IW = Industrial wastewater, pesticide manufacturing
DW = Reagent water

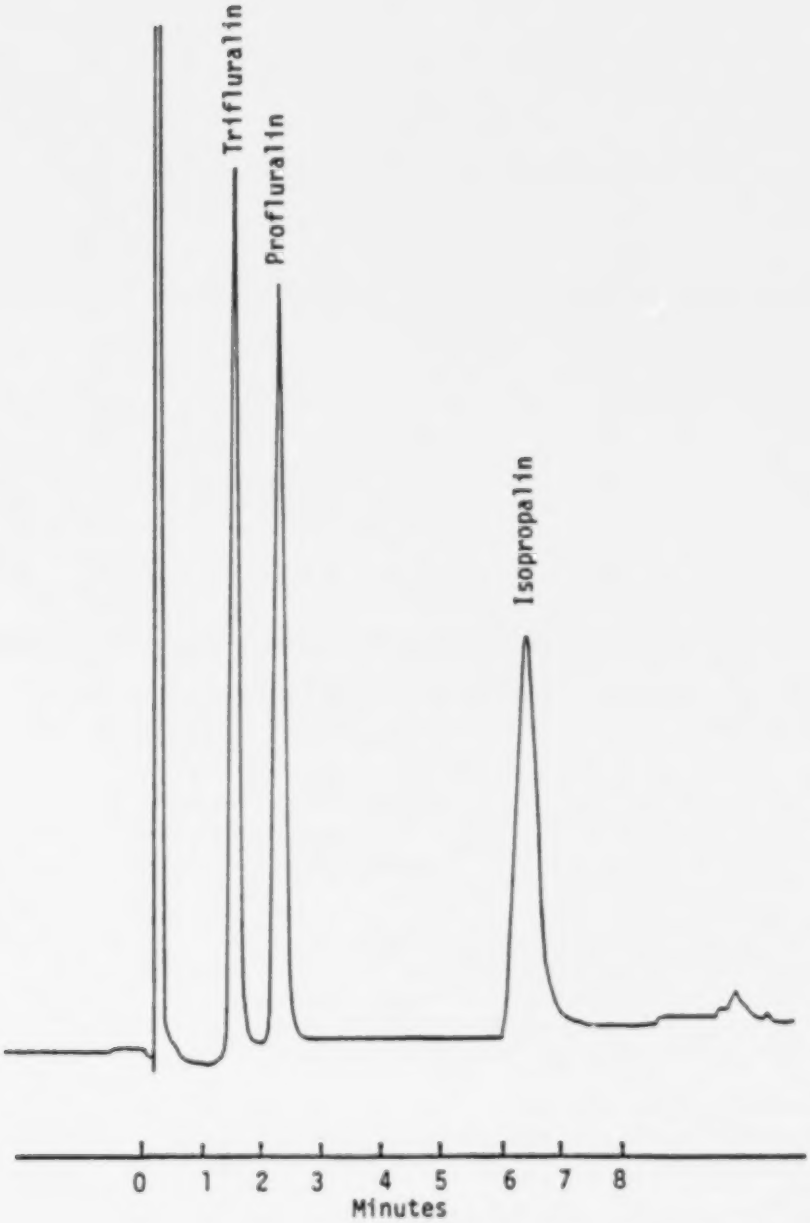

Figure 1. Gas chromatogram of dinitroaniline pesticides on Column 1. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF CYANAZINE IN WASTEWATER

METHOD 629

1. Scope and Application

1.1 This method covers the determination of cyanazine. The following parameter can be determined by this method:

Parameter	STORET No.	CAS No.
Cyanazine	--	21725-46-2

1.2 This is a high performance liquid chromatographic (HLPC) method applicable to the determination of the compound listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The estimated method detection limit (MDL, defined in Section 15) for cyanazine is 6 µg/L. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.5 When this method is used to analyze unfamiliar samples for cyanazine, compound identifications should be supported by at least one additional qualitative technique.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to methanol during concentration to a volume of 10 mL or less. HPLC conditions are described which permit the separation and measurement of cyanazine in the extract by HPLC with a UV detector.[1]

2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [3-5] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with coarse fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Filtration apparatus - As needed to filter chromatographic solvents prior to HPLC.

5.7 Liquid chromatograph - High performance analytical system complete with high pressure syringes or sample injection loop, analytical columns, detector and strip chart recorder. A guard column is recommended for all applications.

5.7.1 Gradient pumping system, constant flow.

5.7.2 Column - 25 cm long x 2.6 mm ID stainless steel packed with Spherisorb ODS (10 µm) or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.7.3 Detector - Ultraviolet, 254 nm. This detector has proven effective in the analysis of wastewaters for cyanazine and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, methylene chloride - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Methanol - HPLC/UV quality.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.6 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. Before use activate each batch at least 16 h at 130°C in a foil covered glass container.

6.7 Stock standard solution (1.00 µg/µL) - A stock standard solution may be prepared from pure standard material or purchased as a certified solution.

6.7.1 Prepare a stock standard solution by accurately weighing approximately 0.0100 g of cyanazine. Dissolve the material in UV quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solution into a TFE-fluorocarbon-sealed screw cap vial. Store at 4°C and protect from light. Frequently check the stock standard solution for signs of degradation or evaporation, especially just prior to preparing calibration standards from it.

6.7.3 The stock standard solution must be replaced after six months or sooner if comparison with a check standard indicates a problem.

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1. The HPLC system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of stock standard to volumetric flasks and diluting to volume with methanol. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should

correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 10 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for cyanazine. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select an internal standard similar in analytical behavior to cyanazine. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels by adding volumes of stock standard to volumetric flasks. To each calibration standard, add a known constant amount of internal standard, and dilute to volume with methanol. One of the standards should be representa- tive of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 10 µL of each calibration standard, tabulate the peak height or area responses against the concentration for both cyanazine and internal standard. Calculate response factors (RF) as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for cyanazine.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of cyanazine in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value is suggested. This procedure[6] determines the adsorption from hexane solution of lauric acid, in mg, per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration. Using stock standard, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s

Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[7]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery of cyanazine does not fall within the control limits for method performance, the results reported for cyanazine in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram as cyanazine, confirmatory techniques such as chromatography with a dissimilar column, or ratio of absorbance at two or more wavelengths must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 It is necessary to exchange the extract solvent to hexane if the Florisil cleanup procedure is to be used. For direct HPLC analysis, the extract solvent must be changed to methanol. The analyst should only exchange a portion of the extract to methanol if there is a possibility that cleanup may be necessary.

10.5 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.6 Pour a measured fraction or all of the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.7 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.8 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane or methanol and a new boiling chip and reattach the Snyder column.

Pour about 1 mL of solvent into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.9 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane or methanol and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with HPLC analysis. If the sample requires cleanup, proceed to Section 11.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of cyanazine for the cleanup procedure is no less than 85%.

11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to cyanazine.

11.2.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.2.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.2.3 Drain the column until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) and with 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2) using a drip rate of about 5 mL/min. These fractions may be discarded. Place a 500-mL K-D flask and clean concentrator tube under the chromatograpy column. Elute the column with 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3), into the K-D flask. Cyanazine elutes quantitatively in Fraction 3.

11.2.4 Concentrate the eluate by standard K-D techniques (Section 10.7), exchanging the solvent to methanol. Adjust final volume to 10 mL with methanol. Analyze by HPLC.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and method detection limit that can be achieved by this method. An example of the separations achieved by this column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 10 µl of the sample extract. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of cyanazine in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of cyanazine injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for cyanazine.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for cyanazine must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[9] The MDL concentration listed in Table 1 was estimated from the response of a 254 nm UV detector to the compound. The estimate is based upon the amount of material required to yield a signal five times the HPLC background noise, assuming a 10-µL injection from a 10-mL final extract of a 1-liter sample.

14.2 In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.[1] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

References

1. "Pesticide Methods Evaluation," Letter Report for EPA Contract No. 68-03-2697. Available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

2. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation, " American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

3. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

4. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

6. ASTM Annual Book of Standards, Part 31, D3086, Appendix X3, "Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid," American Society for Testing and Materials, Philadelphia, PA, p 765, 1980.

7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.

8. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

9. Glaser, J.A. et.al, "Trace Analysis for Wastewaters," Environmental Science & Technology, 15, 1426 (1981).

10. "Determination of Cyanazine in Industrial and Municipal Wastewater," Method 629, EPA No. 600/4-82-010, NTIS No. PB82-156043, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

TABLE 1

CHROMATOGRAPHIC CONDITIONS AND ESTIMATED DETECTION LIMIT

Parameter	Retention Time (min)	Estimated MDL (µg/L)
Cyanazine	10.0	6

Column conditions: Spherisorb ODS (10 µm) packed in a 25 cm long x 2.6 mm ID stainless steel column with a mobile phase flow rate of 1.0 mL/min. Mobile phase: linear gradient from 50% solvent B to 100% solvent B in 2 min, where solvent A is 25% methanol in water and solvent B is 50% methanol in water.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Sample Type	Spike µg/L	No. of Replicates	Average Percent Recovery	Standard Deviation %
Cyanazine	DW	121	7	100.0	8.9
	MW	60.8	7	85.5	3.9
	PW	10100	3	94.3	--
	IW	10100	2	78.0	--

DW = Reagent water.
MW = Municipal wastewater.
PW = Process water, pesticide manufacturing.
IW = Industrial wastewater, pesticide manufacturing.

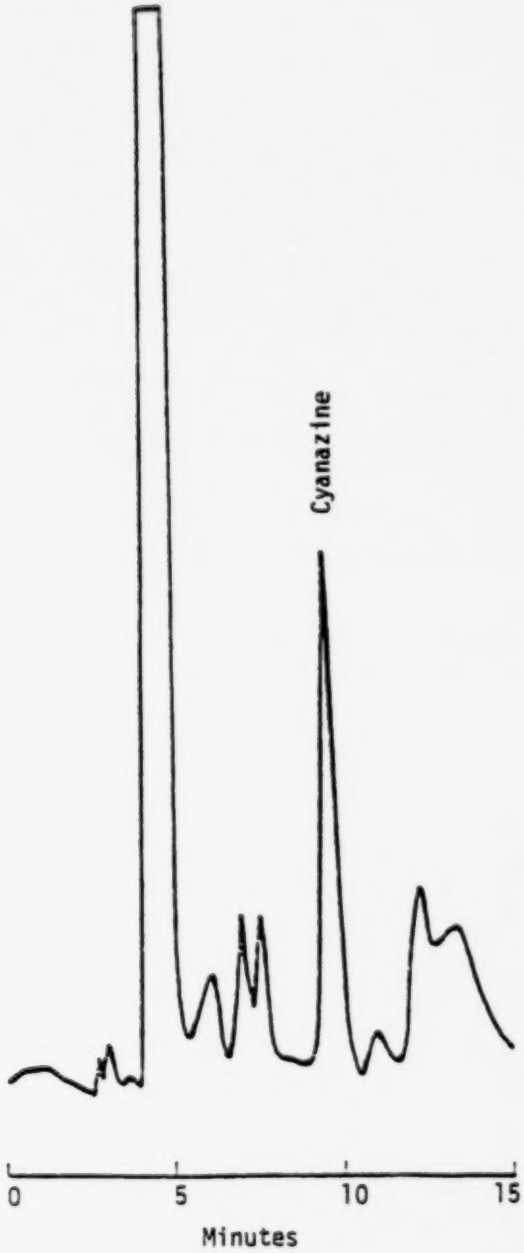

Figure 1. Liquid chromatogram of cyanazine in process water extract on Column 1. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF AOP, BUSAN 40, BUSAN 85, CARBAM-S,
FERBAM, KN METHYL, MANCOZEB, MANEB, METHAM, NABAM
NIACIDE, ZAC, ZINEB, AND ZIRAM
IN WASTEWATER

METHOD 630

1. Scope and Application

1.1 This method covers the determination of dithiocarbamate pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS. No.
Amobam	--	3566-10-7
AOP	--	--
Busan 40	--	--
Busan 85	--	--
Ferbam	--	14484-64-1
KN Methyl	--	--
Mancozeb	--	8065-67-6
Maneb	--	12327-38-2
Metham	--	137-42-8
Nabam	--	142-59-6
Niacide	--	8011-66-3
Polyram	--	9006-42-2
Sodium dimethyldithiocarbamate	--	128-04-1
Thiram	--	137-26-8
ZAC	--	--
Zineb	--	12122-67-7
Ziram	--	137-30-4

1.2 This method fails to distinguish between the individual dithiocarbamates. The compounds above are reduced to carbon disulfide and the total dithiocarbamate concentration is measured. Unless the sample can be otherwise characterized, all results are reported as Ziram. Carbon disulfide is a known interferent.

1.3 This is a colorimetric method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.4 The method detection limit (MDL, defined in Section 12) for maneb, metham and ziram are listed in Table 1. The MDL for a specific dithiocarbamate or wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 This method is restricted to use by or under the supervision of analysts experienced in trace organic analyses. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 liter, is digested with acid to yield carbon disulfide by hydrolysis of the dithiocarbamate moiety. The evolved CS_2 is purged from the sample and absorbed by a color reagent. The absorbance of the solution is measured at 380 and 435 nm using a UV-visible spectrophotometer.[1]

3. Interferences

3.1 Method interferences may be caused by contaminants in reagents, glassware and other sample processing hardware that lead to high blank values and biased results. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] After each use, rinse the decomposition flask and condenser with 4N NaOH and reagent water. Overnight soaking in 4N NaOH may be necessary. Clean the H_2S scrubber between each use with 0.1N HCl in methanol, rinse three times with methanol and bake at 200°C for 15 min. Rinse the CS_2 trap with methanol three times between each use and follow by heating for 15 min at 200°C. Should it become difficult to force the color reagent through the glass frit of the CS_2 trap, clean in the same manner as the H_2S scrubber. After cooling, store glassware sealed to prevent any accumulation of dust or other contaminants.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems.

3.2 Carbon disulfide may be a significant direct interferent in wastewaters. Its elimination or control is not addressed in this method. If correction for background carbon disulfide is required, the CS_2 should be measured by an independent procedure, such as direct aqueous injection gas chromatography.

3.3 Additional matrix interferences may be caused by contaminants that are codistilled from the sample. The extent of matrix interfer-

ences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup provided by the H_2S trap will eliminate or reduce some of these interferences, but unique samples may require additional clean-up approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[3-5] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Dithiocarbamate hydrolysis apparatus (Figure 1) - (Available from Southern Scientific Inc., Box 83, Micanopy, Florida 32267). Apparatus includes the following or equivalent components:

5.2.1 Hot plate with magnetic stirrer.

5.2.2 Hydrolysis flask - 2 L, flat bottom with ground glass joints, 2 necks.

5.2.3 Condenser - low internal volume, ground glass joints, Liebig (Kontes K-447000, 100 mm or equivalent).

5.2.4 Gas washing bottles - 125 mL, with extra-coarse porosity (Kontes K-657750 or equivalent).

5.2.5 Addition funnel - 60 mL, ground glass joint to fit hydrolysis flask, with long stem to reach at least 2 cm below the liquid level in the hydrolysis flask.

5.2.6 Dust trap (Adapter) - to fit top of addition funnel (Kontes K-174000 or equivalent).

5.2.7 Vacuum source - stable pressure with needle valve for control.

5.3 UV-Visible spectrophotometer - Double beam with extended cell path length capability of 1.0 and 4.0 cm cells.

5.4 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 gram. The preparation of calibration standards for some dithiocarbamates (e.g. metham) requires the use of a balance capable of weighing 10 µg.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest. Prepare by boiling distilled water 15 min immediately before use.

6.2 Acetonitrile, diethanolamine, methanol - ACS grade.

6.3 Ethanol - 95%.

6.4 Cupric acetate - Monohydrate, ACS grade.

6.5 Hydrochloric acid - Concentrated.

6.6 Hydrochloric acid, 0.1 N in methanol - Slowly add 8.3 mL conc. HCl to methanol and dilute to 100 mL.

6.7 Sodium hydroxide, 4 N - Dissolve 16 g ACS grade NaOH pellets in reagent water and dilute to 100 mL.

6.8 Stannous chloride - $SnCl_2 \cdot 2H_2O$, ACS grade.

6.9 Zinc acetate solution, 20% - Dissolve 20 g ACS grade $Zn(C_2H_3O_2) \cdot 2H_2O$ in reagent water and dilute to 100 mL.

6.10 Color reagent - Add 0.012 g cupric acetate monohydrate to 25 g diethanolamine. Mix thoroughly while diluting to 250 mL with ethanol. Store in amber bottle with TFE-fluorocarbon-lined cap.

6.11 Decomposition reagent - Dissolve 9.5 g stannous chloride in 300 mL conc. hydrochloric acid. Prepare fresh daily.

6.12 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.12.1 Prepare a stock standard solution for ziram by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in acetonitrile and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.12.2 Transfer the stock standard solution into a TFE-fluorocarbon-sealed screw cap vial. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.12.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

6.12.4 When using other dithiocarbamates for calibration, such as maneb or metham, it may be necessary to weigh microgram amounts of the pure material into small aluminum foil boats and place them directly in the hydrolysis flask.

7. Calibration

7.1 Use ziram as the standard for total dithiocarbamates when a mixture of dithiocarbamates is likely to be present. Use the specific dithiocarbamate as a standard when only one pesticide is present and its identity has been established.

7.2 With the apparatus assembled and reagents in place, Section 10, pour 1500 mL of reagent water into each decomposition flask, add 30 mL of decomposition reagent and start aspiration.

7.3 Spike the water in each flask with an accurately known weight of dithiocarbamate standard. Use a series of weights equivalent to 5-200 µg of CS_2. Follow the procedure outlined Section 10.

7.4 Prepare calibration curves at a minimum of three concentrations by plotting absorbance vs. weight of dithiocarbamate. A separate curve is prepared from readings taken at 435 nm and at 380 nm for each cell path length used. Normally the 435 nm curve is used for calibration above 30 µg ziram (4 cm cell), and the 380 nm curve is used for calibration below 30 µg ziram. The choice of which curve to use is left to the discretion of the analyst. It is recommended that the curves be transformed into mathematical equations using linear least squares fit for the data from 435 nm and quadratic least squares fit for data from the 380 nm.

7.5 The working calibration curve must be verified on each working shift by the measurement of one or more calibration standards. If the response varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured.

8.2.2 Add the known amount of dithiocarbamate standard to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results.

Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 1, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3\,s$$
$$\text{Lower Control Limit (LCL)} = R - 3\,s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[6] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[6]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 11.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[7] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be analyzed within seven days of collection.

10. Sample Analysis

10.1 Assemble the hydrolysis apparatus as follows (See Figure 1):

10.1.1 Place the hydrolysis flask on the hot plate.

10.1.2 Place the addition funnel in one of the necks of the hydrolysis flask and the dust trap in the top of the funnel.

10.1.3 Place the condenser in the other neck and attach two gas washing bottles in succession to the condenser outlet.

10.1.4 Attach a vacuum line with a flow valve to the second scrubber .

10.2 Allow the sample to warm to room temperature. Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the 2-liter hydrolysis flask. Rinse the bottle four times with 100-mL aliquots of reagent water, adding the washes to the hydrolysis flask. Bring the volume in the hydrolysis flask to approximately 1,500 mL with reagent water.

10.3 Place 15.0 mL of color reagent into the CS_2 trap (second gas washing bottle). Place 9 mL of zinc acetate solution into the H_2S scrubber (first gas washing bottle). Add 2 mL of ethanol to the H_2S scrubber. Place a magnetic stirring bar in the hydrolysis flask and place the flask on the hot plate/magnetic

stirrer (ambient at this time). Assemble the apparatus providing adequate support for all glassware. The addition funnel stem opening must be below the water level. Ground glass joints may be slightly coated with silicone grease.

10.4 Start the stirrer, begin water flow through the condenser, and turn on hot plate and begin heating the flask. Open the needle valve slightly and start the aspirator. By closing the needle valve, adjust the airflow through the absorption train until the proper flow is attained. (The column of bubbles extends to the bottom of the spherical expansion chamber at the top of the CS_2 trap.) Add 30 mL of decomposition reagent to the flask. (Note: The analyst must ensure that the sample pH is less than 2 during hydrolysis.)

10.5 Bring the liquid in the flask to a gentle boil. Continue the boiling for 60 minutes, then remove the heat. Continue aspiration until boiling ceases.

10.6 Transfer the contents of the CS_2 trap into a 25.0 mL volumetric flask by forcing the liquid through the glass frit and out of the inlet arm with pressure from a large pipet bulb. Ensure quantitative transfer by rinsing the trap three times with ethanol. Bring the colored solution to volume with ethanol. Mix thoroughly and allow the color to develop for at least 15 min but not more than two h before determining the absorbance.

10.7 Determine the absorbance of the sample at 435 nm and 380 nm using a 1-cm cell or a 4-cm cell as necessary. Determine the weight of dithiocarbamate from the appropriate calibration curve prepared in 7.4.

10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL. If a smaller measured aliquot of sample was used to remain within the range of the color reagent, this step may be omitted.

11. Calculations

11.1 Determine the concentration of total dithiocarbamates in the sample as ziram directly from the calibration curve. When a specific dithiocarbamate is being measured, quantitate in terms of the selected pesticide.

11.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

11.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in 8.3, data for the affected parameters must be labeled as suspect.

12. Method Performance

12.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[8] the MDL concentrations listed in Table 1 were determined using wastewater, and are expressed in concentration units of the spiked materials.[1]

12.2 In a single laboratory, Environmental Science and Engineering, using spiked wastewater samples, the average recoveries presented in Table 1 were obtained. The percent standard deviation of the recovery is also included in Table 1.[1] All recoveries are based on calibrations using the specific dithiocarbamate instead of ziram.

References

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9. "Determination of Dithiocarbamate in Industrial and Municipal Wastewater," Method 603, EPA No. 600/4-82-011, NTIS No. PB82-156050, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

TABLE 1

METHOD PERFORMANCE

Parameter	Method Detection Limit (µg/L)	Sample Type*	Number of Replicates	Spike (µg/L)	Mean Recovery (%)	Standard Deviation (%)
Maneb	15.3	1	7	31.5	97.1	15.5
Metham	3.7	2	7	20.1	94.5	5.9
		3	7	250	65.2	2.8
Ziram	1.9	4	8	32.2	100	2.0
		5	8	1050	96.2	10.0

*Sample type:
1 = Municipal wastewater.
2 = Mixture of 13% industrial (pesticide manufacturing) wastewater and 87% municipal wastewater.
3 = Industrial wastewater, pesticide manufacturing.
4 = Mixture of 40% industrial and 60% municipal wastewater.
5 = 7% industrial process water, 7% industrial wastewater, 86% municipal wastewater.

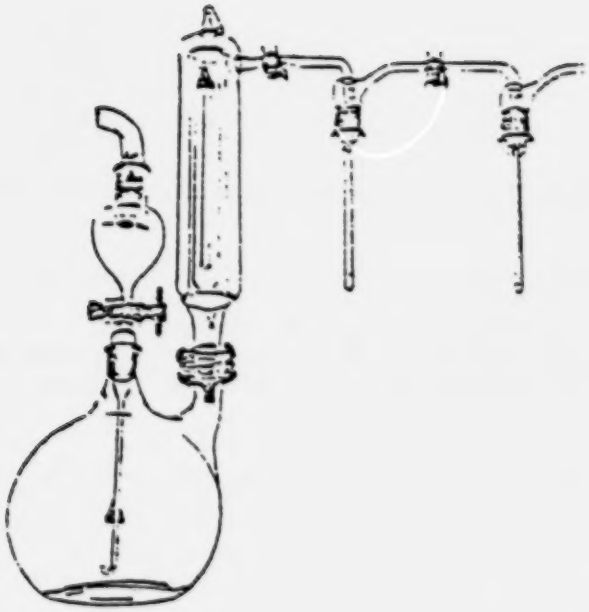

Figure 1. Dithiocarbamate hydrolysis apparatus.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BENOMYL AND CARBENDAZIM IN WASTEWATER

METHOD 631

1. Scope and Application

1.1 This method covers the determination of benomyl and carbendazim. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Benomyl	--	17804-35-2
Carbendazim	--	10605-21-7

1.2 Benomyl cannot be determined directly by this method. Benomyl is hydrolyzed to carbendazim, and both compounds are measured and reported as carbendazim.

1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.4 The method detection limit (MDL, defined in Section 15) for each parameter is 8.7 µg/L. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for either of the compounds above, compound identifications should be supported by at least one additional qualitative technique.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 liter, is acidified if necessary to hydrolyze benomyl to carbendazim. The total carbendazim is extracted with methylene chloride using a separatory funnel. The extract is dried and exchanged to methanol during concentration to a volume of 10 mL or less. HPLC conditions are described which permit the separation and measurement of total carbendazim in the extract by HPLC with a UV detector.[1,2]

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[3] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require cleanup approaches to achieve the MDL listed in Section 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible

for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [4-6] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 250-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Liquid chromatograph - High performance analytical system complete with high pressure syringes or sample injection loop, analytical columns, detector and strip chart recorder. A guard column is recommended for all applications.

5.6.1 Column - 30 cm long x 4 mm ID stainless steel, packed with μ Bondapak C_{18} (10 μm) or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Detector - Ultraviolet, 254 nm. This detector has proven effective in the analysis of wastewaters and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Methylene chloride, methanol - Pesticide quality or equivalent.

6.3 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.4 Sodium hydroxide solution (10N) - Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.5 Sulfuric acid solution (1+1) - Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.6 Mobile phase - Methanol/water (1+1). Mix equal volumes of HPLC/UV quality methanol and reagent water.

6.7 Stock standard solution (1.00 µg/µL) - The stock standard solution may be prepared from a pure standard material or purchased as a certified solution.

6.7.1 Prepare the stock standard solution by accurately weighing approximately 0.0100 g of pure carbendazim. Dissolve the material in HPLC/UV quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solution into a TFE-fluorocarbon-sealed screw cap vial. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.6.3 The stock standard solution must be replaced after six months or sooner if comparison with a check standard indicates a problem.

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1. The HPLC system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of carbendazim stock standard to volumetric flasks and diluting to volume with methanol. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 10 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for carbendazim. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for carbendazim at each standard concentration. If the relative standard deviation

of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select an internal standard similar to carbendazim in analytical behavior. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels of carbendazim by adding volumes of stock standard to volumetric flasks. To each calibration standard, add a known constant amount of internal standard, and dilute to volume with methanol. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 10 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for carbendazim.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of carbendazim in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration

standards. If the response for carbendazim varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate of either benomyl or carbendazim in methanol 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3\,s$$
$$\text{Lower Control Limit (LCL)} = R - 3\,s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[7]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for benomyl or carbendazim does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagent interferences are under control. Each time

a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram as carbendazim, confirmatory techniques such as chromatography with a dissimilar column, or ratio of absorbance at two or more wavelengths may be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Using a 250-mL graduated cylinder, measure 150 mL of well-mixed sample into a 250-mL Erlenmeyer flask. If benomyl is a potentiality in the sample, continue with Section 10.2. If only carbendazim is to be measured, proceed directly to Section 10.3.

10.2 Carefully add 2 mL of 1+1 sulfuric acid and a TFE-fluorocarbon covered magnetic stirring bar to the sample. Check the sample with wide-range pH paper to insure that the pH is less than 1.0. Stir at room temperature for 16 to 24 h.

10.3 Adjust the sample pH to within the range of 6 to 8 with sodium hydroxide. Pour the entire sample into a 250-mL separatory funnel.

10.4 Add 60 mL methylene chloride to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface

between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.5 Add a second 60-mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.6 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.7 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.8 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.9 Increase the temperature of the hot water bath to 85 to 90°C. Momentarily remove the Snyder column, add 50 mL of methanol and a new boiling chip and reattach the Snyder column. Pour about 1 mL of methanol into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.10 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methanol and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the

extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. Proceed with HPLC analysis.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and method detection limit that can be achieved by this method. An example of the separation achieved by this column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 10 µL of the sample extract. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of carbendazim in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of carbendazim injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for carbendazim.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 If the sample was treated to hydrolyze benomyl, report the results as benomyl (measured as carbendazim). If the hydrolysis step was omitted, report results as carbendazim. Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[9] The MDL concentrations listed in Table 1 were determined by extracting 1000-mL aliquots of reagent water with three 350 mL volumes of methylene chloride.[1]

14.2 In a single laboratory (West Cost Technical Services, Inc.), using reagent water and effluents from publicly owned treatment works

(POTW), the average recoveries presented in Table 2 were obtained.[1] The standard deviations of the percent recoveries of these measurements are also included in Table 2. All results were obtained using the same experimental scale described in Section 14.1.

References

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND
METHOD DETECTION LIMITS

Parameter	Retention Time (min)	Method Detection Limit (µg/L)
Benomyl (as carbendazim)	--	25.0
Carbendazim	8.1	8.7

Column conditions: µ Bondapak C_{18} (10 µm) packed in a 30 cm long x 4 mm ID stainless steel column with a mobile phase flow rate of 2.0 mL/min at ambient temperature.
Mobile phase: methanol/water (1+1)

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Sample Type	Number of Replicates	Spike (µg/L)	Average Percent Recovery	Standard Deviation (%)
Benomyl (as carbendazim)	DW	7	51.5	70	15.5
	MW	7	51.5	78	8.8
	MW	7	103	99	6.4
Carbendazim	DW	7	50	106	5.5
	MW	7	50	117	18.5
	MW	7	100	108	11.3

DW = Reagent water
MW = Municipal wastewater

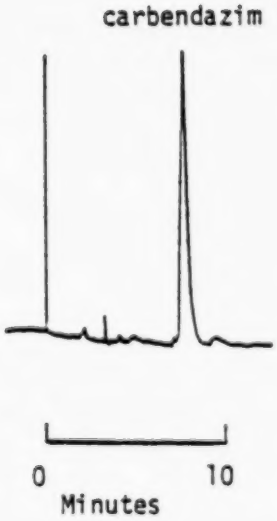

Figure 1. Liquid chromatogram of carbendazim on Column 1. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF CARBOFURAN, FLUOMETURON, METHOMYL, AND OXAMYL IN WASTEWATER

METHOD 632

1. Scope and Application

1.1 This method covers the determination of certain carbamate and urea pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Aminocarb	--	2032-59-9
Barban	--	101-27-9
Carbaryl	39750	63-25-2
Carbofuran	81405	1563-66-2
Chlorpropham	--	101-21-3
Diuron	39650	330-54-1
Fenuron	--	101-42-8
Fenuron-TCA	--	4482-55-7
Fluometuron	--	2164-17-2
Linuron	--	330-55-2
Methiocarb	--	2032-65-7
Methomyl	39051	16752-77-5
Mexacarbate	--	315-18-4
Monuron	--	150-68-5
Monuron-TCA	--	140-41-0
Neburon	--	555-37-3
Oxamyl	--	23135-22-0
Propham	39052	122-42-9
Propoxur	--	114-26-1
Siduron	--	1982-49-6
Swep	--	1918-18-9

1.2 This method cannot distinguish monuron from monuron-TCA and fenuron from fenuron-TCA. Results for the paired parameters are reported as monuron and fenuron respectively.

1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly

permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.4 The method detection limit (MDL, defined in Section 15) for many of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 mL or less. HPLC chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC with a UV detector.[1,2]

2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[3] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware

in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [4-6] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water

to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with coarse fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Flask, round bottom - 500-mL, with standard taper to fit rotary evaporator.

5.2.5 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Rotary evaporator.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Filtration apparatus - As needed to filter chromatographic solvents prior to HPLC.

5.7 Liquid chromatograph - High performance analytical system complete with high pressure syringes or sample injection loop, analytical columns, detector and strip chart recorder. A guard column is recommended for all applications.

5.7.1 Gradient pumping system, constant flow.

5.7.2 Column - 30 cm long x 4 mm ID stainless steel packed with μ Bondapak C_{18} (10 μm) or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.7.3 Detector - Ultraviolet, capable of monitoring at 254 nm and 280 nm. This detector has proven effective in the analysis of wastewaters and was used to develop the method performance statements in Section 14. Alternative

detectors may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, acetonitrile, hexane, methylene chloride, methanol - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.) Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.5 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. Before use activate each batch at least 16 h at 130°C in a foil covered glass container.

6.6 Acetic acid - Glacial.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality acetonitrile or methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1. The HPLC system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile or methanol. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 10 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile or methanol. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 10 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value is suggested. This procedure[7] determines the adsorption from hexane solution of lauric acid, in mg, per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetonitrile or methanol 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Table 2 provides single operator recovery and precision for most of the carbamate and urea pesticides. Similar results should be expected from reagent water for all compounds listed in the method. Compare these results to the values

calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[8] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[8]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be

analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as chromatography with a dissimilar column, or ratio of absorbance at two or more wavelengths may be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[9] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 It is necessary to exchange the extract solvent to hexane if the Florisil clean up procedure is to be used. For direct HPLC analysis the extract solvent must be exchanged to a solvent (either

methanol or acetonitrile) that is compatible with the mobile phase. The analyst should only exchange a portion of the extract to HPLC solvent if there is a possibility that cleanup may be necessary.

10.5 Pass a measured fraction or all of the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate and collect the extract in a 500-mL round bottom flask. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Attach the 500-mL round bottom flask containing the extract to the rotary evaporator and partially immerse in the 50°C water bath.

10.7 Concentrate the extract to approximately 5 mL in the rotary evaporator at a temperature of 50°C. Other concentration techniques may be used if the requirements of Section 8.2 are met.

10.8 Add 50-mL of hexane, methanol, or acetonitrile to the round bottom flask and concentrate the solvent extract as before. When the apparent volume of liquid reaches approximately 5 mL remove the 500-mL round bottom flask from the rotary evaporator and transfer the concentrated extract to a 10-mL volumetric flask, quantitatively washing with 2 mL of solvent. Adjust the volume to 10 mL.

10.9 Stopper the volumetric flask and store refrigerated at 4°C if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap bottles.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the five pesticides listed in Table 3. It should also be applicable to the cleanup of extracts for the other carbamate and urea pesticides listed in the scope of this method.

11.2.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.2.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the volumetric flask to the Florisil column. Rinse the flask twice with 1 to 2 mL hexane, adding each rinse to the column.

11.2.3 Drain the column until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 20% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Place a 500-mL round bottom flask under the chromatography column. Elute the column again, using 200 mL of 6% acetone in hexane (V/V) (Fraction 2), into a second flask. Perform a third elution using 200 mL of 15% acetone in hexane (V/V) (Fraction 3), and a final elution with 200 mL of 50% acetone in hexane (V/V) (Fraction 4), into separate flasks. The elution patterns for five of the pesticides are shown in Table 3.

11.2.4 Concentrate the eluates to 10 mL with a rotary evaporator as described in Section 10.7, exchanging the solvent to acetonitrile or methanol as required.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by this column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7. The standards and extracts must be in the solvent (acetonitrile or methanol) compatible with the mobile phase.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 10 µL of the sample extract. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Calculate and report fenuron-TCA as fenuron and monuron-TCA as monuron. Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[10] The MDL concentrations listed in Table 1 were obtained using reagent water or river water.[2,11]

14.2 In a single laboratory, the average recoveries presented in Table 2 were obtained using this method.[2,11] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Mobile Phase*	Retention Time (Min)	UV Wavelength (nm)	Method Detection Limit (µg/L)
Mexacarbate	A	8.7	254	0.52
Propoxur	A	14.3	280	0.11
Monuron	A	14.4	254	0.003
Carbaryl	A	17.0	280	0.02
Propham	A	17.2	254	0.07
Diuron	A	19.5	254	0.009
Linuron	A	21.0	254	0.009
Methiocarb	A	21.4	254	0.02
Chlorpropham	A	21.8	254	0.03
Barban	A	22.3	254	0.05
Neburon	A	24.3	254	0.012
Propoxur	B	2.0	280	0.11
Methomyl	B	6.5	254	8.9
Carbaryl	B	14.1	280	0.02
Diuron	B	15.5	254	0.009
Linuron	B	17.9	254	0.009
Propoxur	C	1.7	280	0.11
Carbofuran	C	3.5	280	3.2
Fluorometuron	C	3.6	254	11.1
Oxamyl	D	3.2	254	9.2

*Mobile Phase:

A Methanol/1% acetic acid, programmed linearly from 5 to 95% methanol at 2.0 mL/min flow rate and at ambient temperature.

B Acetonitrile/water, programmed linearly from 10% to 100% acetonitrile in 30 min at a flow rate of 2.0 mL/min.

C 50% acetonitrile in water at a flow rate of 2.0 mL/min.

D 35% methanol in water at a flow rate of 2.0 mL/min.

Column: µ Bondapak C_{18} (10 µm) packed in a 30 cm long x 4 mm ID stainless steel column, with a Whatmann Co. PELL ODS (30-38 µm) guard column, 7 cm long x 4 mm ID.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Sample Type*	Spike (µg/L)	No. of Analyses	Average Percent Recovery	Standard Deviation %
Fluorometuron	1	50	7	93.9	7.0
	2	50	7	80.0	7.2
	4	1724	7	99	11.6
Propoxur	1	550	7	94.5	1.7
	3	2200	3	105	3.0
	4	550	7	87.2	7.3
	5	0.5	5	93	6.0
Oxamyl	1	100	7	87	8.4
	2	53	7	84.9	5.5
	2	1080	7	89.8	2.7
Methomyl	1	100	4	74.4	2.4
	3	30660	4	48.2	2.8
	2	100	7	91.8	2.8
	2	1960	7	94.4	1.9
Diuron	1	10	4	89.8	1.0
	3	500	4	56.1	5.0
	2	10	7	90.0	2.5
	2	400	7	95.7	3.2
	5	0.05	5	98	4.7
Linuron	1	10	4	95.0	3.4
	3	4000	4	72.2	5.1
	2	10	7	93.0	1.5
	2	210	7	103	4.6
	5	0.05	5	99	4.7
Carbofuran	1	37	7	87.8	2.7
	4	148	7	99.3	1.4
Barban	5	0.3	5	98	4.1
Carbaryl	5	0.1	5	101	4.1
Chlorpropham	5	0.2	5	95	3.9
Methiocarb	5	0.2	5	95	2.6
Mexacarbate	5	4.0	5	96	3.5
Monuron	5	0.05	5	97	1.7
Neburon	5	0.05	5	96	6.6
Propham	5	0.3	5	88	5.9

* = Sample Type
1 = Reagent Water
2 = Municipal wastewater
3 = Industrial process water, pesticide manufacturing
4 = Industrial wastewater, pesticide manufacturing
5 = River Water

TABLE 3

FLORISIL FRACTIONATION PATTERNS

Parameter	Percent Recovery by Fraction			
	No. 1	No. 2	No. 3	No. 4
Diuron	0	0	24	58
Linuron	0	13	82	0
Methomyl	0	0	0	84
Oxamyl	0	0	92	0
Propachlor	0	94	0	0

Florisil eluate composition by fraction
Fraction 1 - 200 mL of 20% ethyl ether in hexane
Fraction 2 - 200 mL of 6% acetone in hexane
Fraction 3 - 200 mL of 15% acetone in hexane
Fraction 4 - 200 mL of 50% acetone in hexane

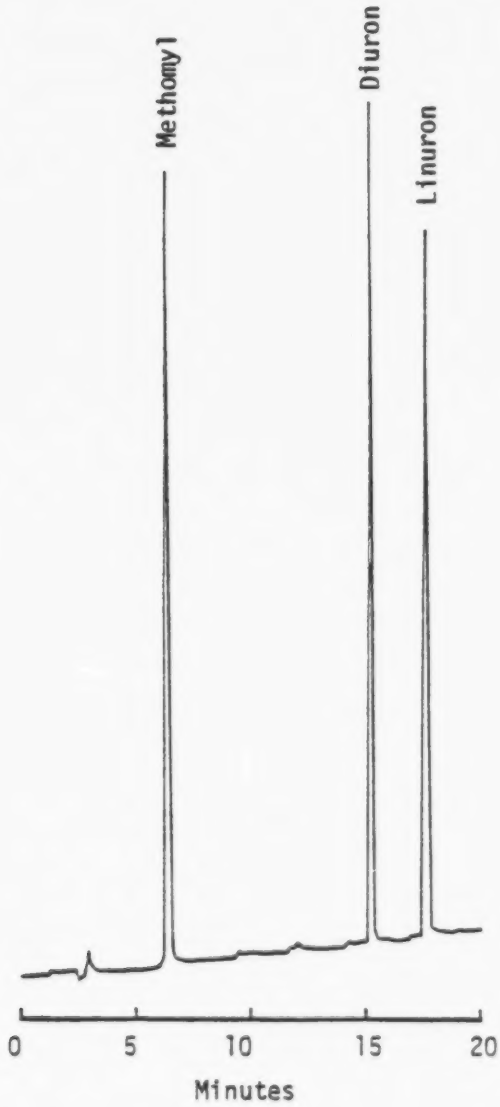

Figure 1. Liquid chromatogram of diuron, linuron and methomyl on Column 1. For conditions, see Table 1.

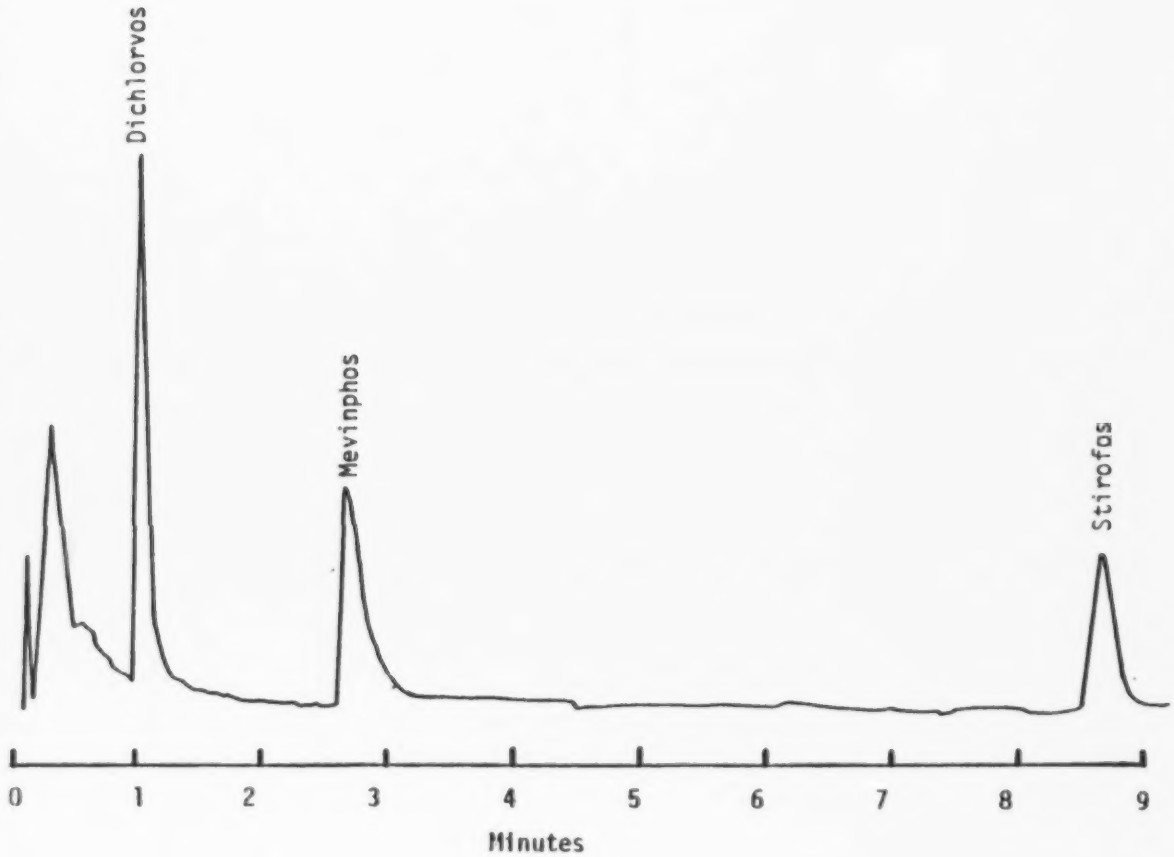

Figure 2. Gas chromatogram of organophosphorus pesticides on Column 1b. For conditions, see Table 1.

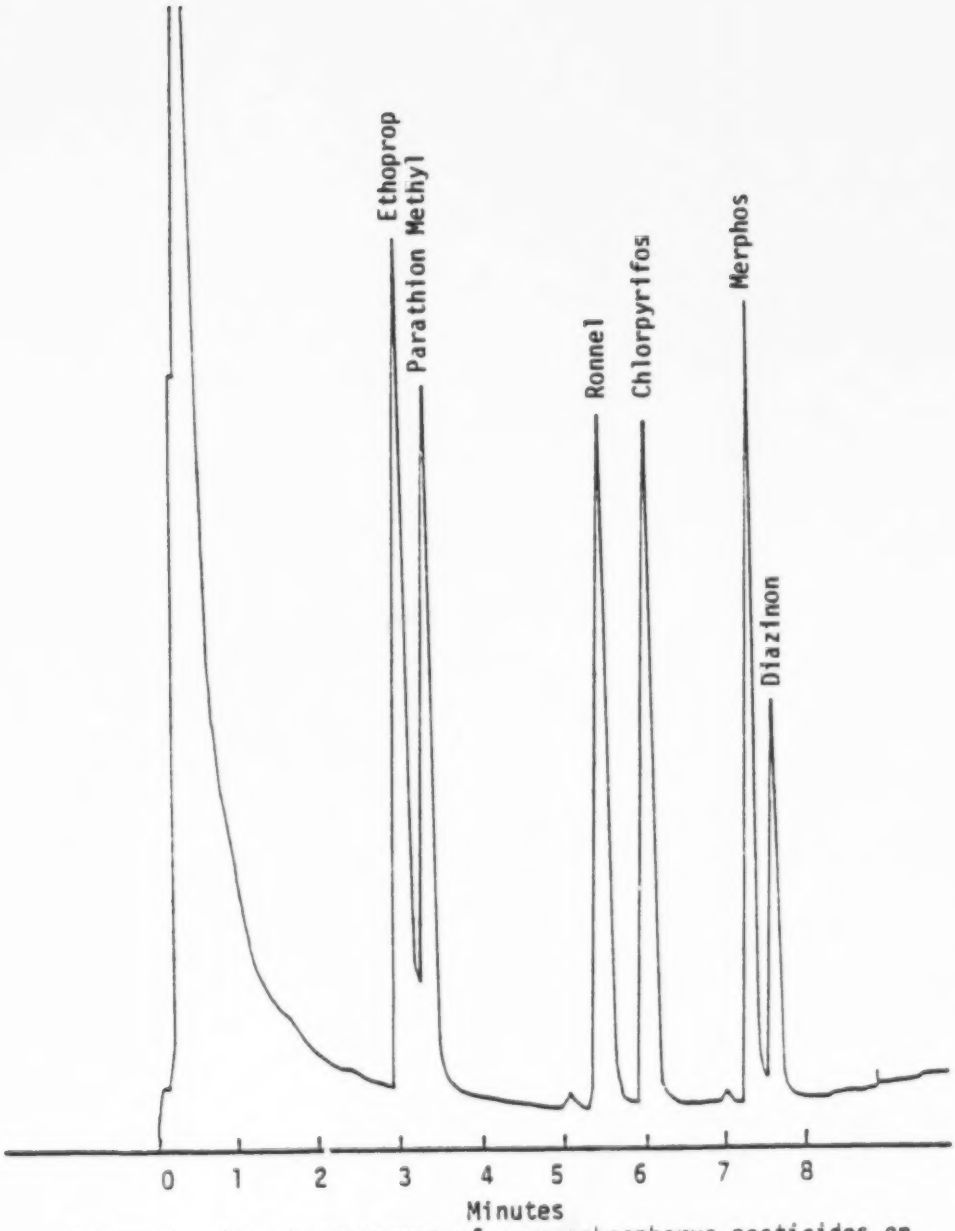

Figure 3. Gas chromatogram of organophosphorus pesticides on Column 2. For conditions, see Table 1.

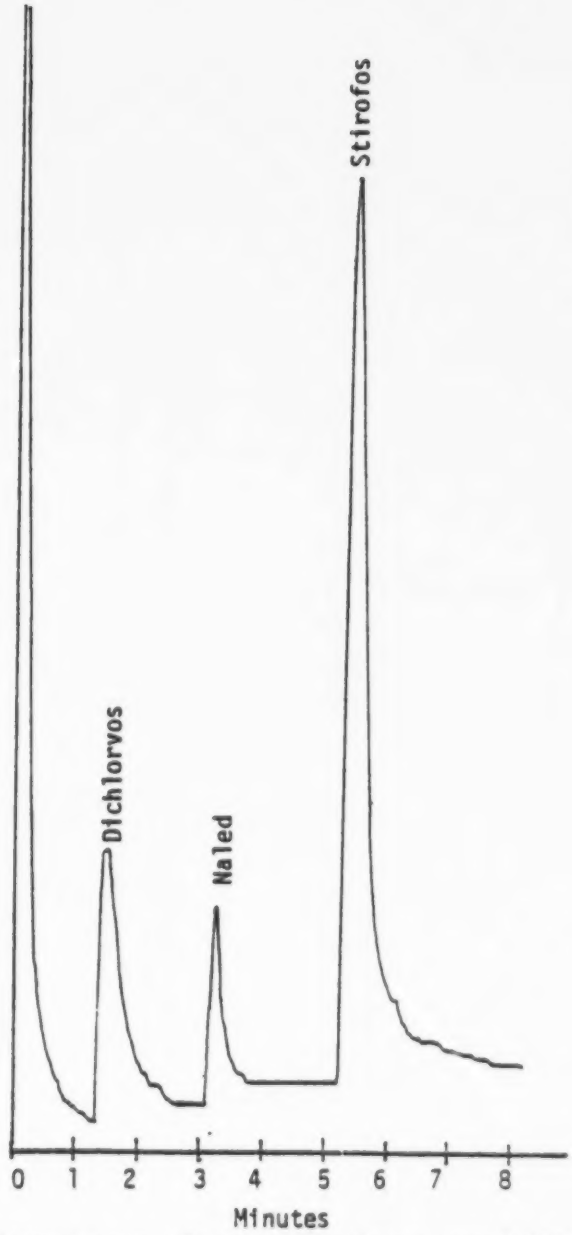

Figure 4. Gas chromatogram of organophosphorus pesticides on Column 3. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF BROMACIL, DEET, HEXAZINONE, METRIBUZIN, TERBACIL, TRIADIMEFON, AND TRICYCLAZOLE IN WASTEWATER

METHOD 633

1. Scope and Application

1.1 This method covers the determination of certain organonitrogen pesticides. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Bromacil	--	314-40-9
Deet	--	134-62-3
Hexazinone	--	51235-04-2
Metribuzin	81408	21087-64-9
Terbacil	--	5902-51-2
Triadimefon	--	43121-43-3
Tricyclazole	--	41814-78-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.3 The method detection limit (MDL, defined in Section 15) for five of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.5 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to acetone during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead detector.[1]

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Thermally stable materials such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require special cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible

for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [3-5] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with TFE-fluorocarbon stopcock, ground glass or TFE stopper.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse fritted disc.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Vials - Amber glass, 10 to 15 mL capacity with TFE-fluorocarbon lined screw cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat at 400°C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 3% SP-2250DB on Supelcoport (100/120 mesh) or equivalent. Operation of this column at high temperatures will seriously reduce its useful period of performance. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with 3% SP-2401 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector - Thermionic bead in the nitrogen mode. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, methylene chloride - Pesticide quality or equivalent.

6.3 Sodium sulfate - (ACS) Granular, anhydrous. Heat treat in a shallow tray at 400°C for a minimum of 4 h to remove phthalates and other interfering organic substances. Alternatively, heat 16 h at 450-500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 h.

6.4 Stock standard solutions (1.00 µg/µL) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.4.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide quality acetone and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.4.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.4.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with acetone. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation

of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetone. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is})/(A_{is}\ C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard in µg/L.
C_s = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[6] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[6]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a

laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[7] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within seven days and completely analyzed within 40 days of extraction.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining

the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Increase the temperature of the hot water bath to about 70°C. Momentarily remove the Snyder column, add 50 mL of acetone and a new boiling chip and reattach the Snyder column. Pour about 1 mL of acetone into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. Analyze by gas chromatography.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a

cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[8] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected in µL.
V_t = Volume of total extract in µL.
V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract in µg.
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS Confirmation

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 s per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[9]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[10]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[11] The MDL concentrations listed in Table 1 were obtained using reagent water.[1]

15.2 In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.[1] The standard deviations of the percent recoveries of these measurements are also included in Table 2.

References

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter	GC Column	Retention Time (Min)	Method Detection Limit (µg/L)
Terbacil	1a	2.1	ND
Bromacil	1a	3.7	2.38
Hexazinone	1a	7.6	0.72
Tricyclazole	1b	3.5	ND
Metribuzin	2a	2.4	0.46
Triadimefon	2a	4.1	0.78
Deet	2b	4.6	3.39

ND = Not determined

Column 1a conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250DB packed in a 180 cm long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature, programmed: initial 210°C, hold for 1 min, then program at 10°C to 250°C and hold. A thermionic bead detector in the nitrogen mode was used to calculate the MDL.

Column 1b conditions: Same as Column 1a, except column temperature isothermal at 240°C.

Column 2a conditions: Supelcoport (100/120 mesh) coated with 3% SP-2401 packed in a 180 cm long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature, programmed: initial 160°C, programmed at injection at 10°C/min to 230°C.

Column 2b conditions: Same as Column 2a, except temperature programmed: initial 130°C, hold for 1 min, then program at 12°C/min to 200°C.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Sample Type	Spike (µg/L)	Number of Replicates	Mean Recovery (%)	Standard Deviation (%)
Bromacil	DW	5	7	92.2	13.9
	MW	11.1	7	89	3.9
	MW	333	7	95	0.8
Deet	DW	5.8	7	99.1	18.4
	MW	5.2	7	92.6	5.9
	MW	515	7	94.2	2.2
Hexazinon	DW	4.9	7	86.6	4.1
	MW	10.1	7	92.2	5.3
	MW	369	7	94.0	1.9
Metribuzin	DW	5.2	6	98.2	2.7
Terbacil	MW	32.8	7	106.7	3.6
	MW	656	7	101	1.2
Triadmefon	DW	5.2	6	126	6.0
	PW	515	4	71.8	4.5
	IW	154.5	7	70.4	3.8
Tricyclazole	MW	12.3	7	69	1.9
	MW	303	7	98	1.2

DW = Reagent water
MW = Municipal wastewater
PW = Process water, pesticide manufacturing
IW = Industrial wastewater, pesticide manufacturing

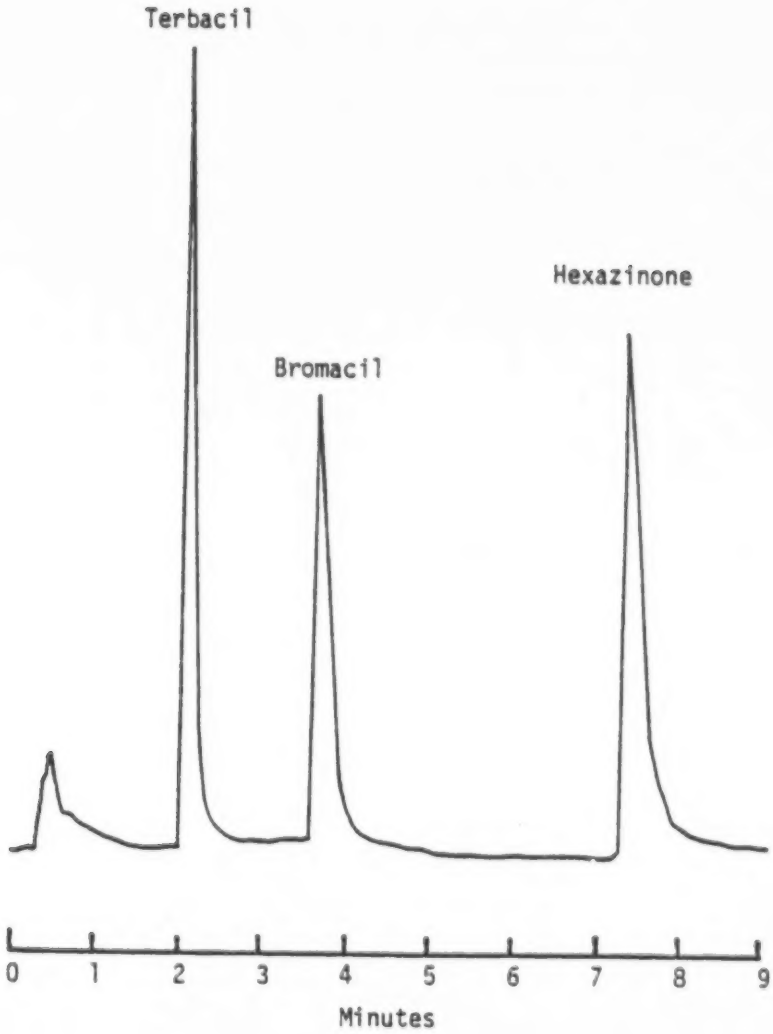

Figure 1. Gas chromatogram of organonitrogen pesticides on Column 1. For conditions, see Table 1.

EPA

United States
Environmental Protection
Agency

Effluent Guidelines Division (WH 552)
Washington, D.C. 20460

Water and Waste Management

TEST METHOD

DETERMINATION OF DICHLOFENTHION, DIOXATHION, AND CARBOPHENOTHION IN WASTEWATER

METHOD 701

1. Scope and Application

1.1 This method covers the determination of certain organophosphorus compounds. The following parameters can be determined by this method:

Parameter	Storet No.	CAS No.
Dichlofenthion	--	97-17-6
Dioxathion	--	78-34-2
Carbophenothion	39786	786-19-6
Diazinon	39570	333-41-5
Methyl Parathion	39600	298-00-0
Malathion	39530	121-75-5
Parathion	39540	56-38-2
Methyl Trithion	--	--
Ethion	39398	563-12-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the above listed compounds in water.

2. Summary of Method

2.1 Water samples (800-900 mL) are extracted with n-hexane. The combined extracts are dried over sodium sulfate and concentrated using a Kuderna-Danish (K-D) apparatus to 0.4 - 0.5 mL. The extract is then cleaned up on a deactivated alumina microcolumn. The eluates are analyzed by gas chromatography with flame photometric detector.

2.2 This method provides a selected cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Any compound or compounds having chemical and physical properties similar to the pesticide of interest may cause interference. The procedure incorporates a column chromatographic technique which eliminates most extraneous material. Special precautions are necessary to avoid contamination during sampling and analysis.

4. Safety

4.1 Follow EPA safety procedure found in Part D of this document.

5. Apparatus and Materials

5.1 Sampling Equipment

Not Available.

5.2 Glassware and Other Equipment

5.2.1 Concentrating apparatus: Kuderna-Danish concentrator, 250-mL capacity with 1-ball Snyder column. Final concentration is performed in the receiver using a 1-ball Snyder micro-column. A calibrated 4.00-mL receiver tube is used with the concentration apparatus.

5.2.2 Cleanup microcolumns: Disposable Pasteur pipets, 15-cm long and 5-mm I.D., are used for the chromatographic cleanup columns. The pipets are washed in warm detergent solution, thoroughly rinsed with dilute hydrochloric acid and organic-free distilled water, then heated to 300°C overnight to remove any traces of organic matter. A column is prepared by plugging the pipet with a small amount of specially cleaned glass wool, adding enough deactivated alumina through a microfunnel to fill 3 cm of the column, followed by another 0.5 cm of anhydrous sodium sulfate.

5.2.3 Separatory funnels: Squibb form, 1- or 2-liter capacity. No lubricant is used on the stopcocks.

5.2.4 Microliter capillary pipets: Volumetric micropipets in 1-, 5-, 10-, and 25-uL sizes; disposable types are satisfactory.

5.2.5 Microliter syringes: Three microsyringes having capacities of 10, 50, and 100 uL, respectively, are used. The syringe needle should be about 2-in long and have a point shaped to prevent punching out a core when penetrating the injection septum.

5.2.6 Volumetric glassware: Class A volumetric flasks in 5-, 10-, and 25-ml sizes. The stoppers should fit well because volatile organic solvents are used for dilutions.

Volumetric ware (supplied by Kontes Glass Co. or equivalent) is acceptable.

5.2.7 Sandbath, fluidized, Tecam, or equivalent.

5.2.8 Microbalance: A Cahn Gram Electro-balance or equivalent.

5.2.9 Integrating equipment: A compensating polar planimeter readable to the nearest 0.001 square inch is acceptable. Other instruments or methods of integration demonstrating greater accuracy may be used.

5.2.10 Recorder: A 1-mv (millivolt) full-scale response, 10-sec (second) pen speed, strip-recorder having a fixed or selectable chart speed of 0.5-in inch per minute is acceptable.

5.3 Gas Chromatograph

5.3.1 Flame-photometric gas chromatograph: A gas chromatograph equipped with a Melpar flame-photometric detector having filters for the specific detection of phosphorus or sulfur. Such an instrument is the Micro-Tec Model MT-220 flame-photometric gas chromatograph. A provision for venting solvent effluent between the column and the detector should be specified.

5.3.2 Gas chromatographic columns: The gas chromatographic columns are fabricated from 1.5-m lengths of Pyrex glass tubing; 1.8-mm I.D. or 4-mm I.D. glass tubing may be used. The smaller bore columns accept injection volumes up to 10 uL and the larger bore columns will accept volumes up to 80 uL. Gas Chrom Q support, 60/80 mesh, is used for the preparation of two different column packings as follows: with 5% by weight DC-200 silicone oil (viscosity 12,500 centistokes), 0.5% by weight QF-1 fluorinated silicone oil (also designed FS 1265), and 0.5% by weight Carbowax 20 M. The packing materials are loaded in the glass columns using vibration and a vacuum to settle. The packing is held in place by small plugs of "silanized" glass wool.

6. <u>Reagents</u>

6.1 Alumina, neutral aluminum oxide, activity grade I, Woelm. Weigh 19 g activated alumina into a 50-mL glass-stoppered Erlenmeyer flask and quickly add 1.0 mL distilled water. Stopper the flask and mix the contents thoroughly by tumbling. Allow 2 hours before use. The deactivated alumina may be used for 1 week.

6.2 Benzene, distilled in glass, pesticide-analysis quality.

6.3 n-Hexane, distilled in glass, pesticide-analysis quality.

6.4 Sodium sulfate, anhydrous granular. Prepare by heating at 300°C overnight; store at 130°C.

6.5 Water, distilled, obtained from a high-purity tin-lined still. Feed water is passed through an activated carbon filter. Distillate is collected in a tin-silver-lined storage tank, and the water is constantly irradiated with ultraviolet light during storage. A gravity delivery system is used, and no plastic material other than Teflon is allowed to contact the distilled water.

7. Calibration

7.1 Establish GC operating parameters equivalent to those indicated in Table 1.

7.2 Calibration Procedure

7.2.1 Weigh 5.00 mg of reference pesticide into a 5.00-mL volumetric flask and dilute to volume with benzene. Make a series of appropriate nanogram standards from this solution. [Example: Take 10.0 uL and dilute to 10.00 mL with the solvent to be used in the analysis. The concentration of pesticide in the resulting solution is 1.0×10^{-9} g/uL, or 1.0 ng/uL (nanogram per microliter)].

7.2.2 A 5.0-uL volume of each of the appropriate standard solutions is injected into the gas chromatograph. The concentration of pesticide in the series of standard solutions should calibrate either the full range of linear detector response or the range of anticipated pesticide concentration in the sample, whichever is less. The injection should be made so that the solution enters the injection port in a single volume and in a reproducible manner. The volume injected should be measured by reading the syringe before and after injection.

7.2.3 All information pertinent to the standardization should be written directly on the recorder chart. Calibration should be performed on both the DC-200 and the QF-1 columns.

7.2.4 During analysis, the standard curve must be checked by running at least two standards at different concentrations so correction can be made for day-to-day fluctuations.

7.2.5 Measurement of gas chromatogram peak areas by use of a planimeter or by any method of equal or greater accuracy is acceptable. If a planimeter is used, the average of at least two measurements is taken as the peak area.

7.2.6 Using log-log graph paper, plot area of response, in square inches (in^2), against nanograms of pesticide injected. Determine the least-squares regression line. The two or more daily response check standards are used to correct the slope of the standard curve, as follows:

$$C = \frac{As}{Ac}$$

where C = correction factor,

Ac = area of check standard obtained from the standard curve, and

As = area obtained from the chromatogram of the check standard.

The slope of the standard curve is corrected by multiplying it by the correction factor.

8. Quality Control

8.1 All glassware, except volumetric flasks, should be washed in the usual manner, rinsed in dilute hydrochloric acid and distilled water, and heat treated at 300°C overnight. Instead of heat treating, the volumetric ware may be solvent rinsed to remove organic matter. A reagent and glassware blank should accompany each analysis.

8.2 See EPA Quality Control procedure found in Part D of this document.

9. Sample Collection, Preservation, and Handling

9.1 Samples should be collected according to the recommended practice for the collection of samples for organic analysis. A 1-liter bottle of water should be collected for each sample. No preservative is used. Samples should be shipped promptly. Unless analyzed within a few days, the water should be protected from light and refrigerated. If the sample contains sediment, the sediment must be analyzed separately. Remove the sediment by centrifugation or filtration through a metal membrane filter.

9.2 See EPA sample collection, preservation, and handling procedure found in Part D of this document.

10. Sample Extraction

10.1 Water samples (800-900 mL) are extracted with n-hexane in such a manner that the water and the container itself are exposed to the solvent. Weigh the uncapped bottle of water on a triple-beam balance and pour the sample into a 1-liter separatory funnel. Allow the bottle to drain for a few minutes, weigh again, and record the weight of water to three significant figures.

10.2 Add 25 mL n-hexane to the empty sample bottle and gently swirl to wash the sides of the container with the solvent. Pour the contents of the sample bottle into the separatory funnel containing the water. Stopper and shake the separatory funnel vigorously for 1 full min, venting the pressure often. Allow the contents to separate for 10 min and draw off the aqueous layer

into the original sample bottle. If the hexane layer emulsifies, separate as much water as possible, then shake the contents of the funnel vigorously so that the liquids contact the entire inside surface of the vessel. (CAUTION: Vent often!) Allow the layers to separate and add approximately 5 mL distilled water to aid the separation, if necessary. Remove the water and pour the extract from the top of the separatory funnel into a 125-mL Erlenmeyer flask containing about 0.5 g anhydrous sodium sulfate.

10.3 Repeat a second and third extraction of the water sample in the same manner using 25 mL n-hexane each time, and collect the extracts in the 125-mL Erlenmeyer flask containing the drying agent. Cover the flask containing the extract with foil and set aside for 30 min.

10.4 Filter the dried extract through glass wool into the Kuderna-Danish apparatus. Add a sand-sized boiling stone and remove most of the hexane by heating on a fluidized sandbath at 100°C in a hood. When the ball in the Snyder column stops bouncing, remove the apparatus from the heat and allow to cool. Add another small boiling stone, fit the receiver with a Snyder microcolumn, and reduce the volume to between 0.4 and 0.5 mL on the sandbath. Set aside to cool. When changing columns, sand must be cleared from the glass joint before opening.

11. Cleanup and Separation

11.1 Quantitatively transfer the contents of the Kuderna-Danish receiver (0.4-0.5 mL) to the top of a deactivated alumina cleanup microcolumn. Use a disposable pipet to transfer. Not more than 0.1-0.2 mL hexane should be needed for washing. Using hexane, elute the extract from the column to a volume of 8.5 mL in a calibrated 10.00-mL receiver. Add only enough hexane so that the solvent level enters the column packing just as the 8.5-mL elution level is reached. Change receivers and continue the elution using 1:1 benzene-hexane solvent. Collect 8.5 mL of eluate in a second receiver. The first fraction of eluate should contain any chlorinated hydrocarbon insecticides, and carbophenthion, methyl trithion, and dichlofenthion. The remaining phosphorus-containing pesticides are eluted in the benzene-hexane fraction. Reduce the volume of each eluate to 1.00 mL using a Kuderna-Danish micro-apparatus on the sandbath. NOTE: The insecticides are separated chromatographically in a predictable order on the micro-column, and this may be used to augment gas chromatographic analysis. Although alumina is the absorbent of choice for the majority of water and sediment samples, occasionally a second pass through a different column is needed for more difficult samples. For a more comprehensive treatment of the cleanup procedure refer to 15.3.

12. Gas Chromatography

12.1 Analyze the eluates by gas chromatography under conditions optimized for the particular gas chromatographic system used.

Run the analysis on the flame-photometric gas chromatographic system on both the DC-200 and the QF-1 columns.

13. Calculations

13.1 The concentration of pesticides in water samples may be determined using the following equation:

$$\text{Concentration of pesticide (ug/L)} = A \times \frac{1}{Cm} \times \frac{Vext}{Vinj} \times \frac{1}{Vs}$$

where A = area of component (in^2)
Cm = corrected slope (in^2/ng)
Vext = volume of extract (mL)
Vinj = volume injected (mL)
Vs = volume of water sample (liters)

This equation may be used to calculate the concentration of pesticides in sediment or soil by substituting the weight of sample in kilograms for the sample volume (Vs) with the resulting concentration expressed as ug/kg.

14. Method Performance

14.1 The results may vary as much as ± 15% for compounds in the 0.01- to 0.10-ug/L concentration range. Recovery and precision data are given in Table 3.

15. References

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15.5 Zweig, G. and Devine, J.M. 1969 Determination of organophosphorus pesticides in water. Residue Rev., v. 26.

15.6 Donald S. Goerlitz and Eugene Brown "Methods for Analysis of Organic Substances in Water," Book 5, Chapter A3 in Techniques of Water Resources Investigations of the United States Geological Survey, 1972.

Table 1

Chromatographic Conditions

Carrier gas flow	75 mL/min nitrogen
Column temperature	185°C
Detector	flame photometric detector -- phosphorus mode

Column--1.8-m x 4-mm I.D. 5% DC-200 or 5% QF-1 on 60/80 mesh Gas Chrom Q

Table 2

Retention Times

Parameter	Relative Retention Time 5% DC-200	5% QF-1
Dioxathion	0.50	0.40
Diazinon	0.55	0.22
Dichlofenthion	0.71	0.34
Methyl Parathion	0.72	1.04
Parathion*	1.00	1.00
Methyl Trithion	2.2	2.7
Ethion	2.6	1.84
Carbophenothion	2.9	2.48

*Parathion retention time: DC-200, 3.82 min; QF-1, 4.55 min

Table 3. Insecticides in water: recovery of compounds added to surface-water samples

Sample No.	Aldrin 0.019	p,p'-DDD 0.080	p,p'-DDE 0.040	p,p'-DDT 0.081	Dieldrin 0.019	Endrin 0.040	Heptachlor 0.018	Heptachlor epoxide 0.021	Lindane 0.021	Malathion 0.181	Methyl parathion 0.082	Parathion 0.076
	Insecticide and amount added (µg/l)											
1	82.0	92.5	88.5	95.0	98.8	95.1	88.8	94.0	90.7	92.9	75.1	99.0
2	113	89.1	94.3	97.0	104	98.0	98.7	94.9	101	106	94.6	96.0
3	90.1	96.0	93.5	103	99.6	88.0	95.2	99.2	99.0	120	89.8	110
4	92.1	95.5	92.1	101	104	81.9	96.3	98.1	107	89.3	81.0	86.0
5	97.0	95.0	93.2	96.0	106	81.1	99.6	103	97.5	105	87.8	107
6	89.5	90.5	92.1	96.0	97.7	83.4	95.5	94.2	109	99.3	86.3	84.1
7	91.2	105	95.6	99.0	104	86.6	95.7	99.1	103	107	81.5	103
8	96.1	99.5	96.8	99.0	105	85.0	103	101	115	109	97.1	118
9	95.7	94.0	99.0	102	103	83.3	100	98.3	101	115	91.7	97.9
10	85.0	94.0	98.3	98.0	103	83.3	93.7	98.3	111	103	99.0	101
11	89.9	93.5	95.6	97.5	99.4	90.0	93.3	92.1	99.8	97.8	96.6	85.7
12	86.5	93.0	89.2	92.6	94.9	83.3	90.0	91.7	94.5	101	83.4	87.8
13	91.9	87.9	87.4	93.6	99.3	89.9	99.2	95.4	94.4	106	86.8	124
14	95.3	89.6	90.7	93.1	105	88.2	97.8	100	98.5	89.0	98.1	86.7
15	85.9	88.6	92.8	93.1	104	89.0	88.1	93.4	108	99.6	86.8	85.4
16	98.4	85.6	92.1	88.6	102	90.6	98.1	97.6	102	105	93.7	89.4
17	96.4	84.1	86.5	92.6	104	92.4	99.8	100	101	100	92.1	95.6
18	84.2	98.5	107	104	110	92.5	88.5	90.6	117	107	121	110
Mean	92.1	92.8	93.4	98.7	102	87.2	95.5	96.7	103	103	91	98.3
Variance	49.3	27.9	24.9	17.1	12.9	23.0	21.5	12.5	51.1	62.4	96.5	139.7
Std. dev	7.02	5.28	4.99	4.14	3.59	4.80	4.64	3.54	7.15	7.96	9.82	11.8
Mean error	−7.9	−7.2	−6.6	−3.3	+2.0	−13	−4.5	−3.3	+3.0	+3.0	−9.0	−1.7
Total error [1]	22	18	13	12	9.2	22	14	10	17	10	29	25

[1] McFarren, E. F., Lisko, R. J., and Parker, J. H., 1970, Criterion for judging the acceptability of analytical methods: Anal. Chemistry, v. 42, p. 358–358.

PART D

QUALITY ASSURANCE REQUIREMENTS

PART D

Quality Assurance Requirements

Part D contains quality assurance (QA) requirements for collection, preservation and handling of samples, quality control (QC), and safety.

Scope and Application

These requirements are designed for applicability to a wide variety of analytical method types. QA for an analytical procedure may vary as a function of method type. However, these requirements are applicable to all nonconventional pesticide methods enclosed herein. The Agency has also presented quality assurance information available from the industry, contractors, and EMSL as shown in methods found in Parts A, B, and C of this document.

QA requirements are presented in three procedures. The collection, preservation, and handling of samples including collecting grab and composite samples are included below under Procedure A. QC is a program developed to assure the quality of data. It involves a rigorous format that includes a control over performance of the laboratory and method analysis. It also includes calibration of instruments, duplication of sample analyses to determine precision, and spiking with a known concentration of compounds to determine percent recoveries and the suitability of the method for the matrix of concern. Details of this program are found in Procedure B below. Laboratory safety procedures involved when using the analytical methods are presented in Procedure C.

Procedure A--Requirements for Sample Collection, Preservation, and Handling

1. Grab samples must be collected in glass containers. Conventional sampling practices should be followed; however, the bottle must not be prerinsed with the sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with program requirements. Automatic sampling equipment must be as free as possible of plastic tubing and other potential sources of contamination.
2. All samples must be adjusted to pH between 5 and 9. When analysis of chlorinated pesticides is to be made it should be preserved with 0.008 percent $Na_2S_2O_3$.
3. The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
4. All samples must be extracted within 7 days and completely analyzed within 40 days of extraction for GC, HPLC, and TLC procedures.
5. All samples must be analyzed within 7 days of collection for colorimetric and titrametric procedures.

Procedure B--Quality Control Requirements

1. The Agency requires that each laboratory operate a formal quality control program. The minimum requirements of this program consist

of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data generated.

(a) Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision. This ability is established as described below.

(b) In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the accuracy and precision procedure.

(c) The laboratory must spike and analyze a minimum of 10 percent of all samples to monitor continuing laboratory performance.

2. To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

(a) Select a representative spike concentration for each compound to be measured.

(b) Add the known amount of standard to each of a minimum of four 1,000-ml aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid.

(c) Calculate the average percent recovery (R) and the standard deviation of the percent recovery (s) for the results. Wastewater background corrections must be made before R and s calculations are performed.

(d) Using average recovery and standard deviation expected for each method parameter, compare these values to R and s. If the data are not comparable, review potential problem areas and repeat the test.

3. The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

(a) Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3s

Lower Control Limit (LCL) = R - 3s

where R and s are calculated as noted above. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

(b) The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control. The accuracy statements should be updated regularly.

4. The laboratory is required to collect in duplicate a portion of its samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10 percent of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified. The laboratory should monitor the frequency of data qualified to ensure that it remains at or below 5 percent.
5. Before processing any samples, the analyst must demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
6. It is recommended that the laboratory adopt additional quality assurance practices for use with these method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on a chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

Procedure C--Safety Requirements

1. The toxicity or carcinogenicity of each reagent used in a method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.
2. Primary standards of toxic compounds should be prepared in a hood, especially when classified or tentatively classified as known or suspected human or mammalian carcinogens.
3. For those compounds classified as potential explosives the following precautions must be followed:
   (a) Use only a well ventilated hood--do not breathe vapors.
   (b) Use a safety screen.
   (c) Use mechanical pipetting aides.
   (d) Do not heat above 90°C--EXPLOSION may result.
   (e) Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers--EXPLOSION may result.
   (f) Store away from alkali metals--EXPLOSION may result.
   (g) Solutions may decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

References

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3. "Determination of Organochlorine Pesticides in Industrial and Municipal Wastewater," Method 608.1, EPA No. 600/4-82-003, NTIS No. PB82-155979, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

4. "Determination of Organophosphorus Pesticides in Industrial and Municipal Wastewater," Method 614, EPA No. 600/4-82-004, NTIS No. PB82-155987, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

5. "Determination of Chlorinated Herbicides in Industrial and Municipal Wastewater," Method 615, EPA No. 600/4-82-005, NTIS No. PB82-155995, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

6. "Determination of Organohalide Pesticides & PCB's in Industrial and Municipal Wastewater," Method 617, EPA No. 600/4-82-006, NTIS No. PB82-156001, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

7. "Determination of Triazine in Industrial and Municipal Wastewater," Method 619, EPA No. 600/4-82-007, NTIS No. PB82-156019, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

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10. "Determination of Cyanazine in Industrial and Municipal Wastewater," Method 629, EPA No. 600/4-82-010, NTIS No. PB82-156043, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

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12. "Determination of Benomyl and Carbendazim in Industrial and Municipal Wastewater," Method 631, EPA No. 600/4-82-012, NTIS No. PB82-156068, January 1982, National Technical Information Center, 5285 Port Royal Road, Springfield, VA 22165.

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APPENDICES

Appendix A

Definition and Procedure for the Determination of the Method Detection Limit

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero and determined from analysis of a sample in a given matrix containing analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

   (a) The concentration value that corresponds to an instrument signal/noise ratio in the range of 2.5 to 5. If the criteria for qualitative identification of the analyte is based upon pattern recognition techniques, the least abundant signal necessary to achieve identification must be considered in making the estimate.
   (b) The concentration value that corresponds to three times the standard deviation of replicate instrumental measurements for the analyte in reagent water.
   (c) The concentration value that corresponds to the region of the standard curve where there is a significant change in sensitivity at low analyte concentrations, i.e., a break in the slope of the standard curve.
   (d) The concentration value that corresponds to known instrumental limitations.

   It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent water (blank), prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated MDL. (Recommend between 1 and 5 times the estimated MDL.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated MDL, proceed to Step 4.

If the measured concentration of analyte is less than the estimated MDL, add a known amount of analyte to bring the concentration of analyte to between one and five times the MDL. In the case where an interference is coanalyzed with the analyte:

If the measured level of analyte is greater than five times the estimated MDL, there are two options:

(1) Obtain another sample of lower level of analyte in same matrix if possible.
(2) The sample may be used as is for determining the MDL if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the MDL and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If blank measurements are required to calculate the measured level of analyte, obtain separate blank measurements for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically deirable to evaluate the estimated MDL before proceeding with 4a. This will: (1) prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an incorrect MDL can be calculated from data obtained at many times the real MDL even though the background concentration of analyte is less than five times the calculated MDL. To insure that the estimate of the MDL is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower MDL. Take two aliquots of the sample to be used to calculate the MDL and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in the desirable range for determining the MDL, take five additional aliquots and proceed. Use all seven measurements to calculate the MDL.
(2) If these measurements indicate the sample is not in the correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 \Big/ n\right]$$

$$S = (S^2)^{1/2}$$

where: the x_i, i = 1 to n are the analytical results in the final method reporting units obtained from the n sample aliquots and $\sum_{i=1}^{n} X_i^2$ refers to the sum of the X values from i = 1 to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1,\ 1-\alpha\ =\ .99)}(S)$$

where:

MDL = the method detection

$t_{(n-1,\ 1-\alpha\ =\ .99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence limits for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (X^2/df) and calculated as follows:

$$MDL_{LCL} = 0.69\ MDL$$
$$MDL_{UCL} = 1.92\ MDL$$

where MDL_{LCL} and MDL_{UCL} are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimated MDL and calculated MDL of subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimated MDL in Step 1, take the MDL as calculated in Step 6, spike in the matrix at the calculated MDL and proceed through the procedure starting with Step 4.

(b) If the current MDL determination is an iteration of the MDL procedure for which the spiking level does not permit qualitative identification, report the MDL as that concentration between the current spike level and the previous spike level which allows qualitative identification.

(c) If the current MDL determination is an iteration of the MDL procedure and the spiking level allows qualitative identification, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F ratio.

$$\text{if } \frac{S_A^2}{S_B^2} < 3.05$$

then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{1/2}$$

if $\frac{S_A^2}{S_B^2} > 3.05$, respike at the last calculated MDL and process the samples through the procedure starting with Step 4.

(c) Use the S_{pooled} as calculated in 7b to compute the final MDL according to the following equation:

$$MDL = 2.681\ (S_{pooled})$$

where 2.681 is equal to $t_{(12,\ 1-\alpha\ =\ .99)}$.

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from percentiles of the chi squared over degrees of freedom distribution.

$$MDL_{LCL} = 0.72\ MDL$$
$$MDL_{UCL} = 1.65\ MDL$$

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

Reporting

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to

determine the MDL must also be identified with the MDL value. Report the mean analyte level with the MDL. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, report the mean recovery, and indicate if the MDL determination was iterated.

If the level of the analyte in the sample matrix exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

Reference

Glaser, J. A., Foerst, D. L., McKee, G. D., Quave, S. A., and Budde, W. L., "Trace Analysis for Wastewaters," *Environmental Science and Technology, 15,* 1426 (1981).

Table of Students' t Values at the 99 Percent Confidence Level

Number of Replicates	*Degrees of Freedom (n-1)*	$t_{(n-1,\ 1-\alpha\ =\ .99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
∞	∞	2.326

APPENDIX B

ABBREVIATIONS, ACRONYMS, AND OTHER TERMS USED IN THIS DOCUMENT

Act - The Clean Water Act

Agency - The U.S. Environmental Protection Agency

AOP - Ambam oxidation product

BAT - The best available technology economically achievable under Section 301(b)(2)(E) of the Act

BBTAC - 1,1′-(2-Butenylene)bis(3,5,7-triaza-1-azo niaadamantane chloride)

Cf - Confirmation

Clean Water Act - (CWA) The Federal Water Pollution Control Act Amendments of 1972 (33 U.S.C. 1251 et seq.) as amended by the Clean Water Act of 1977 (Public Law 95-217)

Conventional Pollutants - For the pesticide industry conventional pollutants are defined as BOD, TSS, and pH.

ECD - Electron capture detector

Effluent Limitations - Any restrictions established by a state or the Administrator on quantities, rates, and concentrations of chemical, physical, biological, and other constituents which are discharged from point sources into navigable waters, the waters of the contiguous zone, or the ocean, including schedules of compliance

FID - Flame ionization detector

FPD - Flame photometric detector

GC - Gas chromatography

GC/MS - Gas chromatography/mass spectrometry

HPLC - High pressure liquid chromatography

Nonconventional Pollutants - For the pesticide industry nonconventional pollutants are defined as nonpriority pollutant pesticides, COD, ammonia, and manganese

Nonconventional Pesticide Pollutants - All pesticides active ingredients which are included under the scope of effluent limitations and standards for BAT, NSPS, and Pretreatment Standards but were not defined as "toxic" under the Clean Water Act.

Non-toxic Pollutants - All conventional or nonconventional pollutants which are currently regulated or proposed to be regulated

NPD - Nitrogen phosphorus detector

NPDES permit - A National Pollutant Discharge Elimination system permit issued under Section 402 of the Act

PCP - Pentachlorophenol

Pesticide - Any technical grade ingredient used for controlling, preventing, destroying, repelling, or mitigation any pest

Pesticide Active Ingredient - The ingredient of a pesticide which is intended to prevent, destroy, repel, or mitigate any pest. The Active ingredients may make up only a small percentage of the final product which also consists of binders, fillers, diluents, etc.

Pesticide Industry - The combined facilities which manufacture as well as formulate and/or package pesticides.

Point Source - Any discernable, confirmed and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged. This term does not include return flow from irrigated agriculture.

Pretreatment Standards - Any restrictions established by the states or the Administrator on quantities, rates and concentrations of chemical, physical, biological and other constituents which are discharged to Publically Owned Treatment Works.

Priority Pollutants - See Toxic Pollutants

QA - Quality assurance. In this document quality assurance pertains to requirements for sample collection, preservation, and handling; quality control; and safety

QC - Quality control

SPM - Spectrometry

308 Survey - A questionnaire drafted by EPA, approved by the National Agricultural Chemicals Association and the Office of Management and Budget (OMB #158-R0160), and subsequently distributed to pesticide manufacturers in July 1978. The primary purpose of the survey was to obtain basic data concerning manufacturing, disposal, and treatment, as well as potential sources of toxic, conventional, and nonconventional pollutants.

TLC - Thin layer chromatography

TLV - Threshhold limit value

Toxic Pollutants - Those 65 pollutants and classes of pollutants specified as an outgrowth of the 1976 Settlement Agreement and declared by Congress to be "toxic" under Section 307(a) of the Clean Water Act of 1977.

TTN - Titration

TWA - Time weighted average

UV - Ultraviolet absorbance

ZAC - Zinc ammonium carbamate

Verification Program - A sampling and analysis project conducted by private contractors to the Agency at selected plants in the pesticide industry. The purpose of the program was to verify the presence of the toxic, conventional, and nonconventional pollutants identified during the screening program and to determine the levels of these pollutants present in process wastewaters prior to and after application of the various control and treatment technologies employed in the industry.

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